Modelización del ciclo fenológico reproductor del olivo (*Olea europaea* L.)

Tesis Doctoral

Universidad de Córdoba

José Antonio Oteros Moreno

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DEPARTAMENTO DE BOTÁNICA, ECOLOGÍA Y FISIOLOGÍA VEGETAL

UNIVERSIDAD DE CÓRDOBA

TESIS DOCTORAL

Modelización del ciclo fenológico reproductor del olivo (*Olea europaea* L.)

Autor

José Antonio Oteros Moreno

Directoras

Carmen Galán Soldevilla - Herminia García Mozo

Córdoba, enero de 2014

TÍTULO DE LA TESIS
Modelización del ciclo fenológico reproductor del olivo (*Olea europaea* L.).

DOCTORANDO
José Antonio Oteros Moreno

INFORME RAZONADO DE LAS DIRECTORAS DE LA TESIS

El documento presentado por el doctorando D. José Antonio Oteros Moreno, y que lleva por título "Modelización del ciclo fenológico reproductor del olivo (*Olea europaea* L.)" corresponde a su trabajo de tesis doctoral. Éste trabajo se ha enfocado en el estudio del ciclo fenológico reproductor del olivo y el desarrollo de modelos de predicción de los principales eventos del ciclo reproductor del olivo, en base a datos fenológicos y aerobiológicos de polen. Los principales eventos analizados fueron: la intensidad de la floración, cuyo estudio y modelización se presenta en los capítulos I y II, "*Biometeorological and autoregressive indices for predicting olive pollen intensity*" y "*Year clustering analysis for modelling olive flowering phenology*"; la evolución del desarrollo floral en el capítulo III "*Modelling olive phenological response to weather and topography*"; y la producción de fruto en el capítulo IV "*Better prediction of Mediterranean olive production using pollen-based models*".

El presente trabajo supone una aportación importante a los campos de la Fenología y la Aerobiología, contribuyendo a mejorar el conocimiento sobre la biología reproductora del olivo, una especie de gran interés agronómico a nivel mundial. Ésta tesis se presenta en formato de Tesis Internacional constituida por un conjunto de cuatro trabajos publicados por el doctorando, como primer autor, en revistas incluidas en el primer y segundo cuartil según el Journal Citation Report (JCR). El doctorando cumple con los requisitos académicos y científicos exigidos para la defensa de la tesis y para poder optar al grado de Doctor en Biología con mención Internacional. Para ello el doctorando ha realizado una estancia de investigación de tres meses en la Universidad de Perugia (Italia). Así mismo, los artículos que comprende la presente tesis han sido publicados en revistas de elevado Factor de Impacto en sus áreas. Finalmente el doctorando presenta otros trabajos publicados, o en vías de publicación, durante su doctorado.

Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, 16 de enero de 2014

Firma de las directoras

Fdo.: Carmen Galán Soldevilla Fdo.: Herminia García Mozo

INFORME SOBRE APORTACIONES DERIVADAS DE LA TESIS DOCTORAL Y FACTOR DE IMPACTO DE LAS REVISTAS CIENTÍFICAS (JOURNAL CITATION REPORTS)

Publicaciones en revistas científicas (Capítulos de la Tesis)

Oteros, J., García-Mozo, H., Hervás-Martínez, C., Galán, C. (2013). Biometeorological and autoregressive indices for predicting olive pollen intensity. International Journal of Biometeorology. 57, 307-316. **(CAPÍTULO I DE LA TESIS)**

Factor de Impacto: 2.59. Posición de la revista en relación a su categoría específica: 62/210; segundo cuartil (Q2), primer tercil (T1)

Oteros, J., García-Mozo, H., Hervás-Martínez, C., Galán, C. (2013). Year clustering analysis for modelling olive flowering phenology. International Journal of Biometeorology. 57: 545-555. **(CAPÍTULO II DE LA TESIS)**

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Oteros, J., García-Mozo, H., Vázquez, L., Mestre, A., Domínguez-Vilches, E., Galán, C. (2013). Modelling olive phenological response to weather and topography. Agriculture Ecosystems & Environment. 179:62-68. **(CAPÍTULO III DE LA TESIS)**

Factor de Impacto: 2.86. Posición de la revista en relación a su categoría específica: 2/57; primer cuartil (Q1), primer tercil (T1)

Oteros, J., Orlandi, F., García-Mozo, H., Aguilera, F., Ben Dhiab, A., Bonofiglio, T., Abichou, M., Ruiz-Valenzuela, L., Trigo, M., Díaz de la Guardia, C., Domínguez-Vilches, E., Msallem, M., Fornaciari, M., Galán, C. (2013). *Better prediction of Mediterranean olive production using pollen-based models*. Agronomy for sustainable development. DOI 10.1007/s13593-013-0198-x. **(CAPÍTULO IV DE LA TESIS)**

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Università degli Studi di Perugia
Dipartimento di Biologia Applicata
University of Perugia - Department of Applied Biology
Borgo XX Giugno, 74 - 06121 Perugia - Italy

Perugia, 20 dicembre 2012

Il sottoscritto Prof. Bruno Romano afferente al Dipartimento di Biologia Applicata dell'Università degli Studi di Perugia, in qualità di Responsabile scientifico dei relativi laboratori di Aerobiologia Applicata e di Biologia della Riproduzione dichiara:

che lo studente José Antonio Oteros Moreno durante il corso di Dottorato presso l'Università di Córdoba (Spagna), Dipartimento di Botanica, Ecología e Fisiologia con la Prof.ssa Carmen Galan Soldevilla, ha frequentato nel periodo 15 Settembre – 20 Dicembre 2012 le strutture da me coordinate. Durante questo periodo José Antonio Oteros Moreno oltre ad avere frequentato con costanza ed impegno le strutture del Dipartimento di Biologia Applicata di Perugia ha contribuito a sviluppare una ricerca sul tema della biologia riproduttiva dell'olivo sotto la guida del Prof. Marco Fornaciari da Passano e del Ricercatore Dott. Fabio Orlandi con ottimi risultati da un punto di vista scientifico.

Inoltre, lo studente ha realizzato in data 04 dicembre 2012 un seminario dal titolo "University of Cordoba" alla presenza di personale del Dipartimento e studenti.

In fede

DIPARTIMENTO DI BIOLOGIA APPLICATA UNIVERSITA' DEGLI STUDI DI PERUGIA

Prof. Bruno Romano

Responsabile scientifico sez. Botanica Ambientale ed Applicata, Dipartimento di Biologia Applicata

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A mis padres

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1. RESUMEN

Resumen

El olivo (*Olea europaea* L.) es la principal especie arbórea con interés agronómico en el área Mediterránea. Esta especie posee un ciclo reproductor que muestra variaciones que responden tanto a su propia genética como al clima. La presente tesis tiene como objetivo el análisis detallado y modelización del ciclo reproductor del olivo y su respuesta a diferentes variables ambientales en el Sur de la Península Ibérica. Para ello este trabajo se basa en el estudio de una base de datos fenológicos, aerobiológicos y meteorológicos que abarcan 30 años en la provincia de Córdoba (Andalucía, España), que es la segunda provincia productora de aceite de oliva de Andalucía, principal región productora en el mundo. Un conocimiento más profundo sobre los principales factores que controlan las variaciones anuales de la floración y producción de fruto de esta especie es de gran interés no sólo a nivel agronómico, sino también médico, dado el carácter alergógeno de sus granos de polen, y ecológico, ya que el acebuche, *Olea europaea* var. *sylvestris* Brot.**,** es un arbusto característico y bioindicador del ecosistema mediterráneo. Aunque gran parte de la tesis se centra en el comportamiento y respuesta fenológica del olivo en la provincia de Córdoba, el capítulo IV de esta tesis aborda un estudio global sobre la producción de aceituna en el área Mediterránea, analizando datos de Andalucía (España), Italia y Túnez.

Se han analizado estadísticamente las relaciones que mantienen diferentes factores ambientales con los aspectos más críticos del ciclo reproductor del olivo (la intensidad de la floración, la fenología floral y la producción de fruto). A partir de éste análisis se han desarrollado modelos descriptivos y predictivos del ciclo reproductor, estudiando desde sus primeras fases hasta la producción final de cosecha, con un gran interés científico y una alta aplicabilidad y transferencia a la sociedad, ya que permite la predicción de importantes eventos biológicos, como fechas, duración e intensidad de la floración, así como la producción de fruto con varios meses de antelación.

En los capítulos I y II, "*Biometeorological and autoregressive indices for predicting olive pollen intensity*" y "*Year clustering analysis for modelling olive flowering phenology*", se analizan las características de la intensidad de la floración,

expresada en esta especie anemófila mediante su Índice Polínico. En el primer capítulo se propone la construcción de índices explicativos de la variabilidad anual de la intensidad polínica en olivo, mientras que en el segundo se realiza un análisis cluster que agrupa los años con características meteorológicas y fenológicas similares y discrimina las variables independientes que más influyen sobre la fenología floral, y especialmente sobre la intensidad de floración.

En el capítulo I, se ha modelizado la producción de polen a partir de índices bioclimáticos y autorregresivos que explican la influencia y control de diferentes variables ambientales. Gracias a la metodología diseñada para el desarrollo de los índices biometeorológicos, se ha permitido analizar el papel que juegan los eventos meteorológicos extremos sobre la floración. También ha sido desarrollado un índice autorregresivo que ha permitido conocer la importancia de la propia dinámica del ciclo reproductor del olivo. Para el desarrollo de modelos matemáticos sobre la intensidad de la floración en el capítulo II se ha diseñado una metodología integradora denominada "*three-step method*" que consiste en realizar una agrupación, mediante una técnica *clustering*, una clasificación, mediante redes neuronales artificiales, y finalmente una modelización mediante regresión por el método de los mínimos cuadrados parciales. Aplicando esta metodología se ha observado que los parámetros ambientales que más afectan y controlan a intensidad de la floración en la provincia de Córdoba son la precipitación durante el periodo de prefloración y la temperatura durante el mes de marzo. Así mismo se han generado modelos de predicción efectivos para la intensidad de la emisión polínica, y por tanto útiles en la prevención de los síntomas de la alergia al polen de olivo.

En el capítulo III "*Modelling olive phenological response to weather and topography*", se han estudiado los parámetros ambientales que afectan al desarrollo de todas las fases de la fenología floral del olivo en la provincia de Córdoba. Se han identificado qué condiciones topográficas y meteorológicas determinan la respuesta de las diferentes poblaciones y variedades de olivo en esta provincia. Las variables topográficas que más influyen sobre la fenología reproductora son la altitud y la orientación Este-Oeste de la máxima pendiente de la ladera y los parámetro meteorológicos más importantes para el desarrollo fenológico son la temperatura durante invierno y primavera temprana y la disponibilidad hídrica durante el proceso de desarrollo de los órganos reproductores.

Finalmente, en el capítulo IV "*Better prediction of Mediterranean olive production using pollen-based models*", se analiza a nivel de la Cuenca Mediterránea los parámetros determinantes en la producción final de fruto en Andalucía, principal región productora de España, en Italia y en Túnez, teniendo en cuenta la intensidad de la floración y la meteorología como variables principales. Para la modelización de la producción de cosecha se han empleado datos de los principales centros productores de aceite de oliva del mundo con la finalidad de obtener conclusiones más globales acerca de la caracterización bioclimatológica del olivo y, de esta forma, poder desarrollar modelos de predicción regionales y globales útiles en la Cuenca del Mediterráneo.

Los resultados obtenidos en la presente tesis contribuyen tanto a una mejor caracterización bioclimatológica del olivo, como a profundizar en el conocimiento de la respuesta de esta especie a las condiciones ambientales, y especialmente sobre la biología de su ciclo reproductor. Éstos resultados han sido obtenidos gracias al desarrollo e implementación de nuevas y específicas metodologías estadísticas, que resultarán muy útiles en futuros estudios, tanto en éstas como en otras zonas olivareras, e incluso como base aplicable a estudios de modelización fenológica sobre otras especies arbóreas.

Abstract

The olive (*Olea europaea* L.) is the leading commercial tree crop in the Mediterranean area. Its reproductive cycle displays considerable variations due to inherent genetic factors but also to climate response. This thesis provides a detailed analysis and modelling of the olive reproductive cycle and its response to a range of environmental variables in the southern Iberian Peninsula. Analysis was based on phenological, aerobiological and meteorological data recorded over the last 30 years in the province of Córdoba (Andalusia, Spain), the second-largest olive-oil-producing province in Andalusia, which is in turn the world's largest producing region. A more thorough knowledge of the factors governing year-on-year changes in olive flowering and fruit production is clearly of agricultural interest. It is also useful for medical purposes—since olive pollen is highly allergenic—and for ecological reasons, given that the wild olive *Olea europaea* var. *sylvestris* Brot., is a characteristic shrub used as a bioindicator for Mediterranean ecosystem. Although the thesis focuses mainly on the behaviour and phenological response of the olive tree in the province of Córdoba, Chapter IV offers an overview of olive production in the Mediterranean area, drawing on data for Andalusia (Spain), Italy and Tunisia.

A statistical analysis is made of the correlations between various environmental factors and critical features of the olive reproductive cycle (flowering intensity, floral phenology and fruit production). The results are used in the construction of models to describe and predict the reproductive cycle, from the earliest phases through to harvest. These models are of considerable scientific interest and can readily be transferred for social applications, since they enable the prediction—several months in advance—of major biological events such as the timing, duration and intensity of flowering and the volume of fruit production.

Chapters I and II "*Biometeorological and autoregressive indices for predicting olive pollen intensity*" and "*Year clustering analysis for modelling olive flowering phenology"* analyse variables relating to flowering intensity, expressed in this anemophilous species by the Pollen Index. The first chapter focuses specifically on the construction of indices to account for year-on-year variations in olive flowering intensity, while in the second chapter a cluster analysis is used to group years with

similar meteorological and phenological characteristics and to distinguish those variables most influencing floral phenology, and more particularly flowering intensity.

In Chapter 1, pollen production is modelled using bioclimatic and autoregressive indices which account for the influence and impact of a range of environmental variables. The method designed for the construction of these indices enables analysis of the role played by extreme weather events in flowering. The autoregressive index provides crucial information on the dynamics of the olive reproductive cycle. For the mathematical modelling of flowering intensity in Chapter II, a “three-step method” is used, consisting in grouping using a clustering technique, classification using artificial neural networks, and modelling using partial least squares regression. The findings show that the environmental variables most affecting and governing flowering intensity in the province of Córdoba are rainfall during the period prior to flowering and temperature during the month of March. Effective models are also constructed to predict pollen emission intensity; these are especially valuable in preventing symptoms in patients allergic to olive pollen.

Chapter III “*Modelling olive phenological response to weather and topography*” examines the environmental factors affecting each phase of the olive’s floral phenology in the province of Córdoba. The topographical and meteorological variables most influencing the response of different local olive populations and varieties are identified. The topographical factors most affecting reproductive phenology are altitude and East-west orientation of the steepest slope, while the most important weather-related variables are winter temperature, spring temperature and water availability during reproductive organ development.

Finally, Chapter IV “*Better prediction of Mediterranean olive production using pollen-based models*” analyses the factors that determine olive fruit production in the Mediterranean Basin, focussing on Andalusia—Spain’s largest olive-producing region—Italy and Tunisia; for this purpose, for this purpose, flowering intensity and weather-related factors are taken as the major variables Harvest size is modelled using data from the world’s largest olive-oil-producing regions, in order to draw overall conclusions regarding the bioclimatological characterization of the olive, with a view to enabling the construction of regional prediction models applicable to the Mediterranean Basin as a whole.

The results of this thesis help to improve the bioclimatic characterisation of the olive, as well as contributing to our knowledge both of the olive's response to environmental conditions and of the biology of its reproductive cycle. The results were obtained using new, purpose-designed statistical methods which will be useful in future research both in the study area and in other olive-producing regions, and may provide a basis for the phenological modelling of other tree species.

2. INTRODUCCIÓN

2.1 Contexto de la tesis doctoral.

La presente tesis doctoral se centra en un estudio de la fenología floral y la aerobiología del olivo (*Olea europaea* L.), una de las especies más importantes en la Cuenca del Mediterráneo. La mayor parte de la tesis se desarrolla en la provincia de Córdoba, una de las principales áreas olivareras del mundo, aunque parte de la tesis es un estudio global en el área mediterránea incluyendo datos de otras zonas de Andalucía (España), de Italia y de Túnez. El olivo es de especial interés en estas zonas de estudio donde la producción y la comercialización del aceite de oliva es uno de los principales recursos económicos. El estudio de las características reproductoras del olivo tiene una alta relevancia por diferentes motivos. De su producción de fruto depende la de otros productos, destacando el aceite de oliva, con una gran importancia económica. El aceite de oliva es un alimento esencial en la dieta mediterránea, presentando su consumo múltiples efectos beneficiosos para la salud (Papadopoulos y Boskou, 1991; López-Miranda et al., 2010). Recientes estudios evidencian el papel del olivo como buen bioindicador de los efectos del cambio climático en el área mediterránea (Galán et al., 2008; García-Mozo et al., 2010a; Bonofiglio et al., 2013). Sin embargo, la expansión de este árbol en monocultivo ha generado problemas de salud provocados por la alta presencia de su polen, considerándose al polen de olivo como la principal causa de polinosis entre la población local (Barber et al., 2008).

Como objetivo principal de esta tesis se ha planteado caracterizar los requerimientos bioclimáticos del olivo durante todo su ciclo reproductor, combinando disciplinas complementarias, la Aerobiología y la Fenología, a través de un profundo y detallado análisis estadístico de bases de datos históricas para la modelización del comportamiento reproductor del olivo frente a las condiciones ambientales. En los capítulos I y II se ha estudiado la intensidad de la floración mediante un enfoque aerobiológico, en el capítulo III se han analizado las causas externas que guían a la fenología floral mediante el análisis de datos fenológicos de campo y en el capítulo IV se han analizado los principales factores que modulan la producción de fruto.

Los resultados derivados de la presente tesis gozan de aplicabilidad directa y pueden ser transmitidos a corto plazo a la sociedad permitiendo la predicción de importantes eventos biológicos, como fecha, duración e intensidad de la floración, o la producción de cosecha con varios meses de antelación.

2.2 El olivo (*Olea europaea* L.)

2.2.1 Distribución geográfica del olivar.

El olivo (*Olea europaea* L.) pertenece a la familia Oleaceae, una familia de árboles y arbustos característica de regiones templadas y cálidas y que comprende alrededor de 29 géneros. El acebuche (*Olea europaea* var. *sylvestris* Brot.) es uno de los más representativos de la vegetación mediterránea, que tuvo una gran expansión en el norte de la Cuenca del Mediterráneo a partir del Holoceno, hace unos 11800 años. Su área de distribución se incrementó en decremento de *Pinus nigra* J.F.Arnold, tras el último periodo glacial (Carrión et al., 2010). El olivo se empezó a cultivar en Oriente Medio hace unos 6000 años (Barranco et al., 2008; Kaniewski et al., 2012).

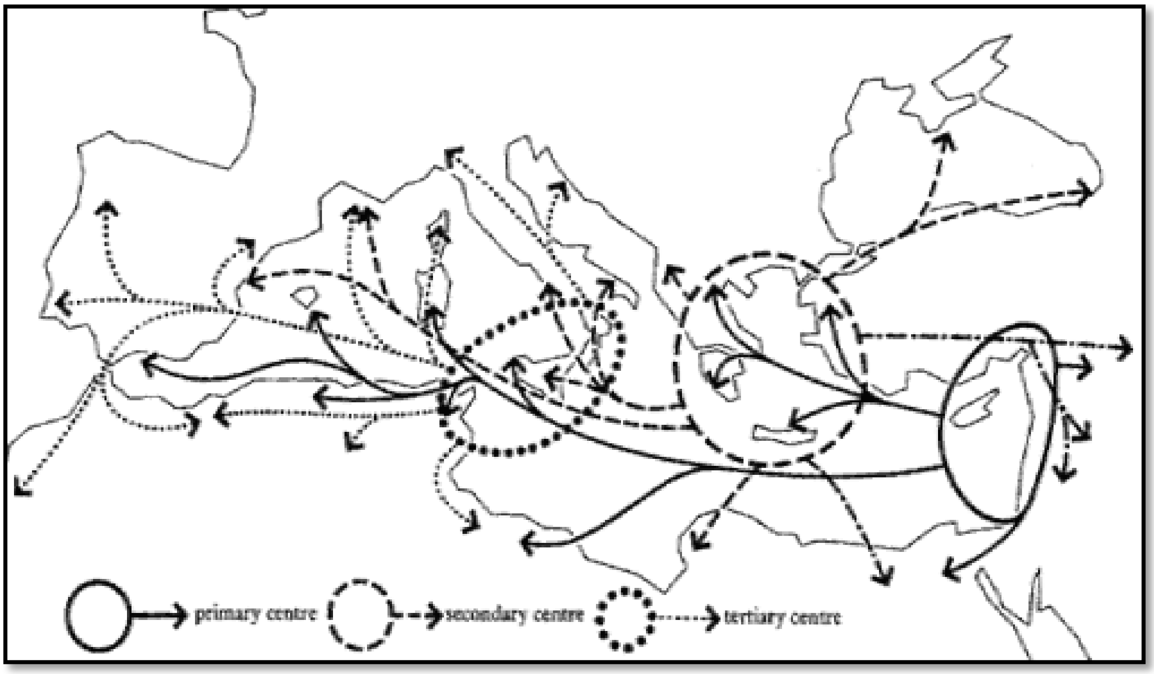

Figura 1. Evolución geográfica del olivo, *Olea europaea L.* (Simmonds, 1976).

Posteriormente, el cultivo se extendió a toda la Cuenca del Mediterráneo. La Figura 1 muestra los tres centros de diversificación principales durante su expansión por la zona (Simmonds, 1976). Recientes estudios han puesto en evidencia altos niveles de polen de olivo en el sustrato de Córdoba durante la época pre-romana, hace aproximadamente 2500 años, lo que pudo deberse a una expansión del cultivo por parte del pueblo íbero o a una expansión del acebuche durante esa época, en cualquiera de los caso debido a la acción antrópica (Martín-Puertas et al., 2009). Posteriormente en la edad Moderna, con el descubrimiento de América, este árbol traspasó el Atlántico, y en la actualidad su cultivo se ha extendido en países como Estados Unidos, Argentina, Chile o Australia, debido a la creciente demanda del producto. Su distribución se restringe entre los 30º y los 45º de latitud, tanto en el hemisferio norte como en el hemisferio sur, aunque el 95% de la producción mundial de aceite de oliva sigue concentrado en el área mediterránea (COI, 2013).

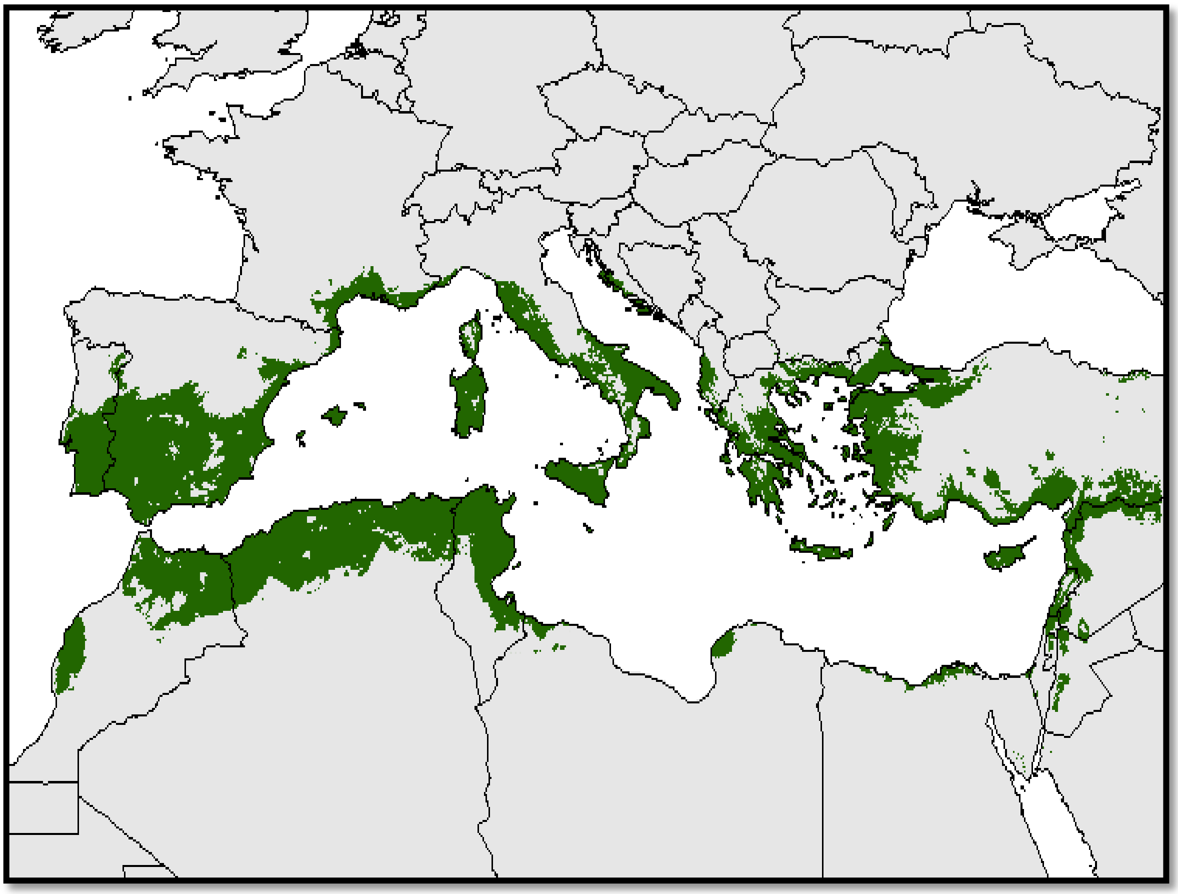

Figura 2. Nicho ecológico de distribución potencial del olivo (*Olea europaea* L.).

En la Figura 2 se puede apreciar el nicho ecológico idóneo para la distribución potencial del olivo. Éste nicho ecológico ha sido calculado mediante un modelo de desarrollo propio de máxima entropía en base a los requerimientos bioclimáticos de esta especie. En primer lugar, se han calculado las condiciones bioclimáticas necesarias para una presencia natural de *Olea europaea* var. *sylvestris,* posteriormente se han modelizado estas características bioclimáticas a escala global para calcular su mapa de distribución potencial, concentrada mayoritariamente en el área mediterránea. Como resultado se observó que más del 90% del área mundial potencialmente idónea para el cultivo del olivo se encuentra en la Cuenca Mediterránea, localizándose la mayor parte de ésta en la zona occidental, si bien también se detectan zonas ecológicas potenciales para esta planta en otros lugares del mundo que comparte similitudes climáticas, como Sudáfrica, Argentina o California. Esta figura muestra la distribución potencial en el área mediterránea, aunque la distribución real depende de diferentes factores, como la acción antrópica en la determinación de la superficie de cultivo, efectos de competencia entre plantas, características edáficas o la escasa variabilidad genética del olivo cultivado (*Olea europaea* var. *europaea* L.) que hace que esté adaptado a un área más restringida.

2.2.2 El cultivo del olivo y su importancia.

La rentabilidad del cultivo del olivo radica principalmente en el aceite de oliva, éste producto representa más del 90% de la salida comercial de las aceitunas, siendo la aceituna de mesa la segunda salida comercial del fruto (Barranco et al., 2008).

En la Figura 3 se observa la evolución de la producción y del consumo de aceite de oliva durante las últimas décadas (COI, 2013). El incremento de la producción mundial de aceite de oliva coincide con la creciente demanda del producto. Éste aumento se ha conseguido gracias a la expansión de la superficie de cultivo, así como al desarrollo de estrategias de densificación, intensificación productiva e irrigación y de mejoras técnicas en las explotaciones. Sin embargo, en el caso de España no se aprecia una relación paralela y sí un estancamiento en el consumo. La tendencia creciente en la producción es debido a la mayor actividad exportadora de nuestro país, la cual ha sido favorecida por la incorporación de España al Mercado Común Europeo.

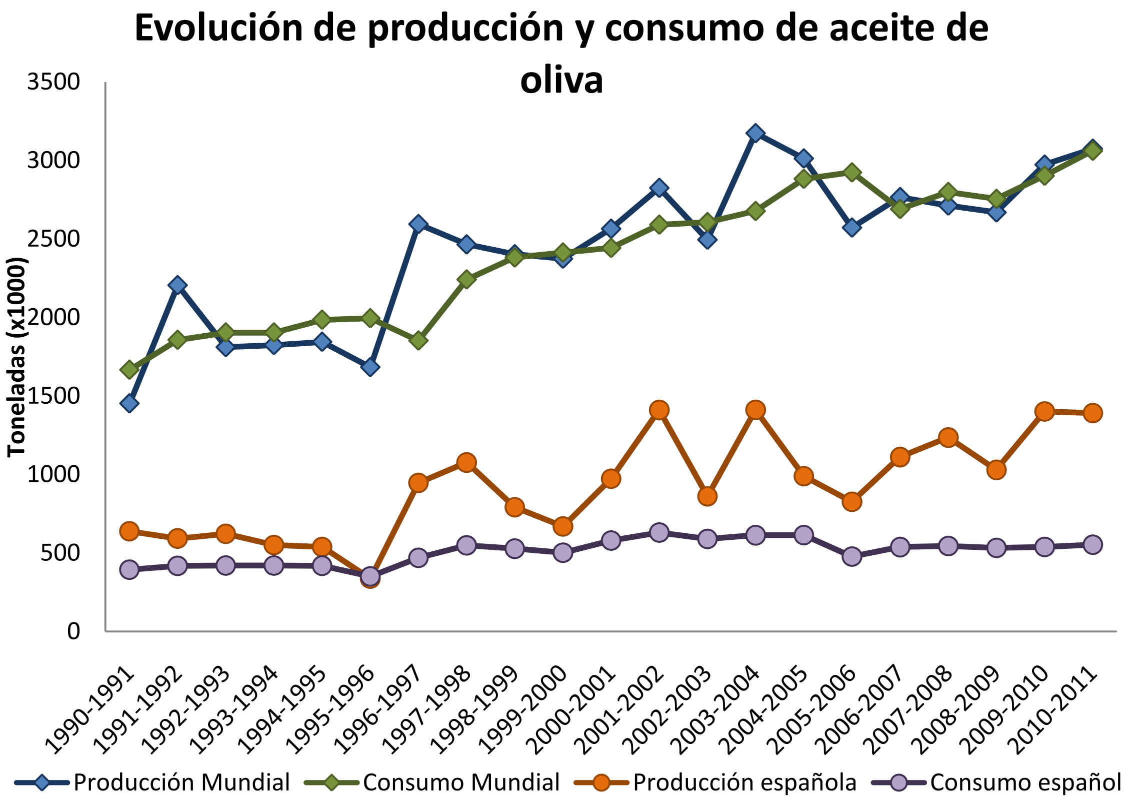

Figura 3. Evolución de la producción y consumo de aceite de oliva.

Según los datos del Centro Oleícola Internacional (COI, 2013), España es el primer país productor de aceite de oliva del mundo, habiendo obtenido en la campaña 2010/2011 cerca del 50% de la producción mundial, seguido por Italia (15%) y Grecia (10%).

Sin embargo, los niveles de consumo mundial sitúan a España como segundo país (25%), tras Italia (30%). Los niveles de exportación sitúan a España e Italia como los dos países principales del mundo, habiendo sido las exportaciones españolas levemente superiores a las italianas durante la campaña 2010/2011 (31% frente al 30% de las exportaciones mundiales). A pesar de estos datos, Italia también es el principal importador mundial acaparando prácticamente la totalidad de la producción de algunos países como Túnez. Grecia, el tercer productor mundial, también es el tercer consumidor mundial (10%), de modo que no aparece en la lista de países exportadores ya que consume toda su producción. Túnez, que tradicionalmente ha sido el cuarto productor mundial y un gran exportador, durante la campaña 2010/2011 fue el tercer país con mayores exportaciones mundiales (17%).

Las perspectivas futuras apuntan hacia un incremento en la producción mundial de aceite de oliva y en su consumo. Se espera un notable incremento en la producción de aceite de oliva debido a la incorporación de nuevas técnicas de producción y al aumento de la superficie de cultivo. Los países del Mediterráneo oriental, principalmente Turquía, lideran las expectativas en el incremento de producción.

2.2.3 El olivar en Andalucía y en Córdoba.

En España, la principal zona productora de aceite de oliva es Andalucía, siendo Jaén la provincia mayor productora de aceite de oliva, seguida por Córdoba (Figura 4). Sevilla es la primera provincia productora de aceituna de mesa.

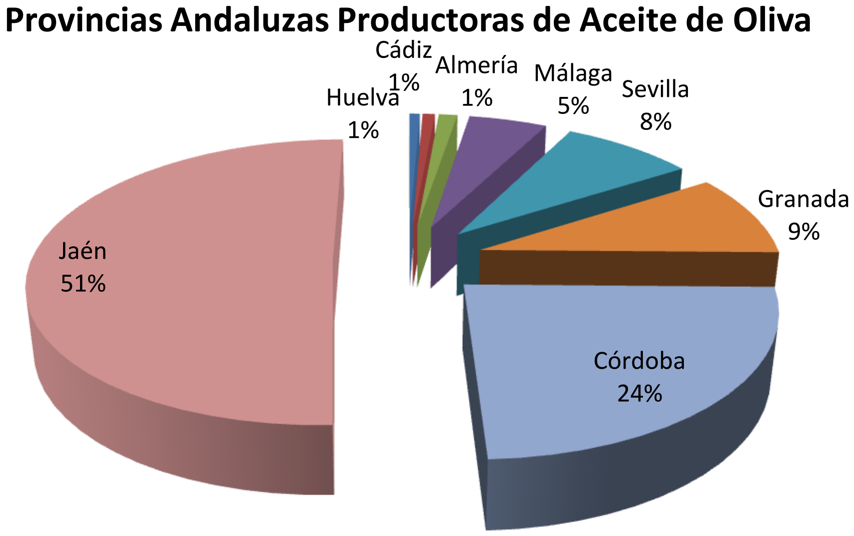

Figura 4. Provincias andaluzas productoras. 2010/2011.

La mayor parte de la presente tesis se centra en el estudio del comportamiento fenológico y aerobiológico del olivo en la provincia de Córdoba. El clima de la ciudad de Córdoba presenta las características del clima mediterráneo con cierta continentalidad. Según los datos proporcionados por la AEMET durante 30 años (1982-2011), la temperatura media anual es de 18,1ºC y la precipitación media anual es de 591 mm, con la posibilidad de grandes cambios interanuales.

Como se ha comentado con anterioridad, Córdoba se encuentra localizada en el área con mayor superficie de cultivo del mundo, Andalucía. En la Figura 5 se aprecia la distribución actual del olivar en Andalucía, representando éste el principal cultivo de la región. Los cultivares más frecuentes en Córdoba son Hojiblanca, Picual y Picudo.

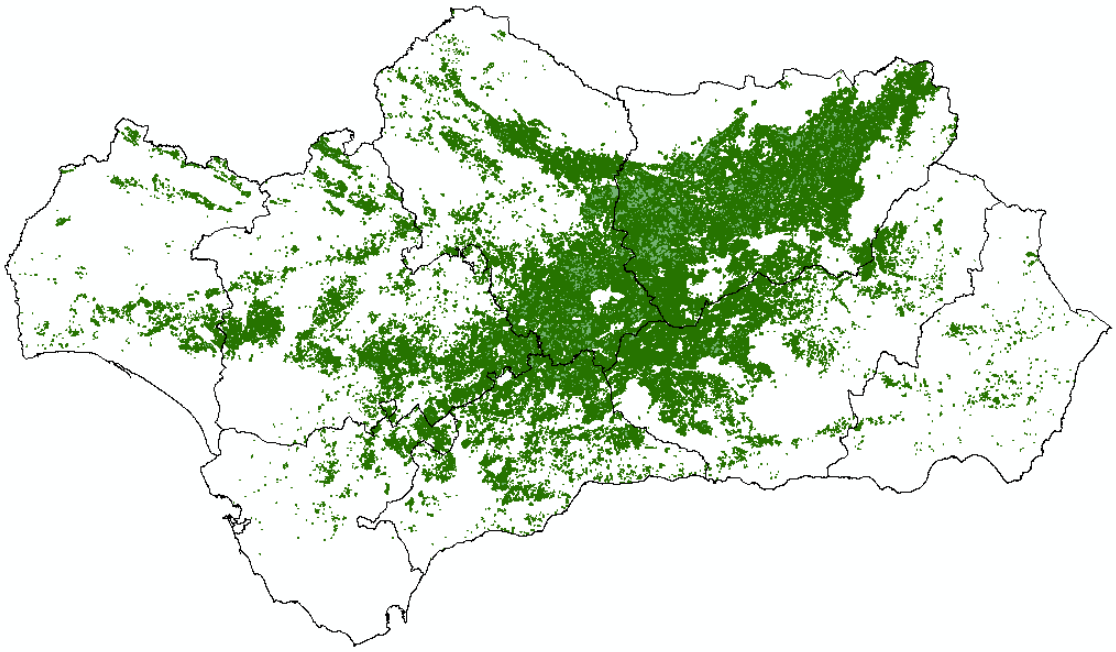

Figura 5. Distribución del olivar en Andalucía (Junta de Andalucía, 2010).

En Andalucía es frecuente observar un paisaje bastante homogéneo característico del núcleo del olivar andaluz. Las zonas olivareras se caracterizan por presentar extensos monocultivos mono-varietales, prácticamente sin la presencia de vegetación adicional, ya que el manejo agrícola tradicional favorece la eliminación de todo material vegetal que no pertenezca al cultivo, denominándose frecuentemente a los olivares como *"mares de olivos";* si bien, la baja biodiversidad que caracteriza a las explotaciones olivareras tradicionales ha llevado a denominarlas también como *"desiertos de olivos".* Por otro lado, es frecuente observar una gran homogeneidad genética dentro de los propios olivares formados por clones de una misma planta (Ipek et al., 2009).

2.2.4 Caracterización biológica del olivo.

2.2.4.1 Aspectos generales.

El olivo es una especie longeva y con una elevada capacidad de adaptación a condiciones cambiantes. Es una especie plástica con gran capacidad para adaptarse a la climatología del Mediterráneo, caracterizada por inviernos suaves y veranos cálidos y secos. Resiste temperaturas de -8ºC, e incluso más bajas siempre que no se prolonguen demasiado, aunque las heladas pueden producir daños en variedades de floración temprana (Aguilar et al., 1995). Altas temperaturas durante el periodo de floración son perjudiciales para la producción de cosecha puesto que pueden conducir a un elevado grado de aborto ovárico (Rallo, 1994), además de producir daños vegetativos (Mancuso et al., 2002; Cansev et al., 2012), mala fecundación (Koubouris et al., 2009) y cambios metabólicos (Malik y Pérez, 2011). Bajas temperaturas pueden así mismo causar daños (Cansev et al., 2011), siendo las temperaturas moderadas las que favorecen la capacidad fotosintética y el proceso de fecundación (Zufferey, 2000; Vuletin-Selak et al., 2013). La pluviosidad de la región del olivo se caracteriza por la relativa escasez de precipitaciones y por la irregularidad de las mismas. Aunque un bajo estrés hídrico mejora el crecimiento vegetativo y reproductor del olivo, éste árbol tolera bastante bien las sequías (Dell'Amico et al., 2012; Martinelli et al., 2012). Aun así, sequías demasiado prolongadas pueden causar daños (Bacelar et al., 2007, 2009; Rapoport et al., 2012; Çamoglu, 2013).

El olivo es un árbol polimorfo que cuenta con una fase juvenil con hojas claramente diferentes de las de la fase adulta, aunque este polimorfismo no es tan notable en los árboles propagados vegetativamente. Se trata de un árbol perennifolio, con tronco generalmente tortuoso. Posee hojas simples, opuestas, de corto peciolo, coriáceas, de contorno elíptico o lanceolado-oblongo, de 2,5 cm a 7,5 cm, penninerviadas con el borde entero y algo revuelto sobre el envés, cara superior de fuerte cutícula y color verdinegro, envés brillante tapizado por pelos lepídotos. Cuenta con inflorescencias en racimos axilares (Barranco et al., 2008).

Su tamaño y su potencial para dar fruto están íntimamente relacionados con las condiciones ambientales. En climas fríos los árboles suelen ser más pequeños que en condiciones de cultivo más cálidas, siempre que el agua no sea un factor limitante. El olivo requiere una elevada intensidad de luz para la diferenciación de los botones florales y el desarrollo de los brotes, por lo que en la mayoría de los cultivares el fruto se localiza en la superficie de la copa. El olivo florece al final de la primavera (abril-mayo) en el hemisferio norte, desarrolla abundante número de inflorescencias con 10-35 flores cada una. El cuajado de fruto es del 1 % al 3% de las flores, caracterizando al olivo su elevado aborto de frutos. El fruto se desarrolla durante el verano y madura durante el otoño (Lavee, 1996).

Aunque la polinización del olivo fue entomófila en origen, hoy en día se considera como mixta, siendo mayoritariamente anemófila (Morettini y Pulselli, 1953). La anemofilia secundaria posiblemente ha sido el fruto del manejo agrícola propiciando la presencia de grandes cantidades de flores en el olivo. En años de baja floración de otras especies frutales, puede apreciarse en el olivo una gran actividad de abejas (Domínguez-Vilches et al., 1993a).

El olivo es parcialmente autoincompatible, lo que quiere decir que favorece la polinización cruzada por otros individuos, facilitando el crecimiento del tubo polínico a mayor velocidad cuando se trata de granos de polen ajenos, frente al propio que crece más lentamente (Ben Amar et al., 2013). El grado de autoincompatibilidad depende del cultivar y de las condiciones ambientales. Muchos investigadores han demostrado que la polinización cruzada aumenta el cuajado y la producción de la mayoría de los cultivares (Farinelli et al., 2004; Spinardi y Bassi, 2012; Zhu et al., 2013).

Las flores se presentan como inflorescencias en racimos axilares (Figura 6). Se compone de cuatro sépalos verdes soldados que forman un cáliz en la base de la flor y cuatro pétalos blancos también soldados por la base. La flor tiene dos estambres, con una antera amarilla grande dividida en dos lóbulos. El fruto del olivo es una drupa en la que se distinguen las siguientes partes: pedúnculo, epicarpio, mesocarpio, endocarpio y embrión (Valdés et al., 1987).

Figura 6. Inflorescencia de olivo.

El grano de polen es isopolar, de simetría radial (Figura 7). Esférico o subcircular, con su eje polar de 22±2 µm de longitud y su eje ecuatorial de 21,6±2,4 µm de longitud (se indica media ± desviación estándar). Tricolporado, con los colpos muy largos y pequeña apocolpia, subterminales (Valdés et al., 1987; Trigo et al., 2008). Tal y como se detalla en el apartado 4.1, es un polen considerado como alergógeno, llegando a ser la principal causa de alergia en algunas zonas de países de clima Mediterráneo (D'Amato et al., 2007; Barber et al., 2008).

Figura 7. Granos de polen de olivo.

2.2.4.2 Ginoesterilidad, partenocarpia y aborto masivo de frutos.

Existen ciertos cultivares que son más propensos a la autopolinización, produciendo frutos partenocárpicos de bajo valor comercial llamados zofairones

(Figura 8). La polinización cruzada reduce la formación de zofairones en algunos cultivares (Fernández-Escobar y Gómez-Valledor, 1985; Barranco et al., 2008; Koubouris et al., 2010).

Figura 8. Diferentes tipos de frutos partenocárpicos (Lavee, 1996).

Otro fenómeno muy característico del olivo es la llamada ginoesterilidad, que consiste en la producción de flores que poseen el gineceo estéril de modo que actúan como flores masculinas imperfectas (Figura 9). Estas flores se desarrollan debido al aborto del ovario en la fase primordial, apreciándose normalmente cierto grado de desarrollo ovárico en ellas. Estas flores producen polen viable (Barranco et al., 2008). Algunos individuos que producen todas sus flores ginoestériles son los llamados “olivos masculinos”, éste fenómeno no es muy frecuente debido a que el manejo agrícola no lo ha propiciado.

Figura 9. Flor perfecta de olivo (izquierda) y flor masculina de olivo (derecha). (Barranco et al., 2008)

Otra característica que define al olivo es el elevado porcentaje de aborto de frutos durante el periodo de cuajado. La alta capacidad competitiva de los jóvenes frutos con otros frutos más atrasados en su maduración, es la causa fundamental de la masiva abscisión de estos últimos que tiene lugar en las semanas que siguen a la floración. En años con buena floración, el cuajado de un 1-2% de las flores es suficiente para obtener una buena cosecha desde el punto de vista comercial. Se considera que una flor perfecta por inflorescencia es suficiente para conseguir una cosecha máxima, por consiguiente, la cantidad de flores ginoestériles no suele estar en relación con la producción. Sin embargo, en casos raros de algunos cultivares, como el Ascolano, la población total de flores masculinas es tan elevada que no llega a producirse un cuajado considerado como comercial. Por otro lado, los frutos partenocárpicos poseen mucha menos capacidad de competencia por recursos que los frutos normales, lo que produce que los zofairones sufran un crecimiento más lento y se presenten usualmente como racimos. (Barranco et al., 2008; Lavee, 1996).

2.2.5 Los ciclos vegetativo y reproductor del olivo.

La estacionalidad anual del clima Mediterráneo durante la evolución de las especies ha provocado que éstas respondan con patrones anuales en sus ciclos de vida como adaptación a los cambios cíclicos del clima. En la mayoría de los casos, los organismos vegetales manifiestan de manera más notoria una estacionalidad anual en sus ciclos fisiológicos que los animales, ya que las plantas no pueden adquirir adaptaciones conductuales.

2.2.5.1 El ciclo vegetativo.

El olivo ha adaptado su crecimiento vegetativo al ciclo climático anual de la Cuenca Mediterránea. Así en el hemisferio norte, el comienzo del crecimiento vegetativo de las yemas apicales y axilares de las ramas más nuevas se produce tras la latencia invernal. A partir de entonces, durante la primavera, la temperatura juega un importante papel. Temperaturas elevadas (a partir de 35ºC) conducen al cierre de estomas, lo que impide el intercambio gaseoso y la fotosíntesis, deteniéndose el crecimiento. De este modo, durante la época estival, el crecimiento se ralentiza. En el último periodo de crecimiento, durante el otoño y previo al letargo invernal, el grado de crecimiento se suele ver favorecido por precipitaciones abundantes.

Esta tendencia anual de doble pico en el crecimiento del árbol que aparece en zonas con clima Mediterráneo, se transforma en una campana de Gauss en el caso de climas más suaves, donde el crecimiento primaveral es más lento pero continuo, alcanzando su pico en la época estival, mientras que en el periodo otoñal se produce una disminución del mismo por el descenso más brusco de temperaturas. Por tanto, el crecimiento vegetativo del árbol está íntimamente relacionado con la temperatura y la disponibilidad de agua, además de las distintas técnicas de cultivo que se apliquen. Entre los signos más evidentes de crecimiento vegetativo se encuentra el tamaño de las hojas y la longitud de los entrenudos (Lavee, 1996; Barranco et al., 2008).

Temperaturas extremas y déficit hídrico pueden producir importantes daños y detienen el crecimiento vegetativo (Mancuso et al., 2002; Cansev et al., 2011, 2012; Bacelar et al., 2007, 2009; Rapoport et al., 2012).

2.2.5.2 El ciclo reproductor.

En cuanto al ciclo de crecimiento reproductor, sin embargo, éste se desarrolla de manera bienal (Figura 10). Así pues, la fructificación se deriva de procesos acontecidos durante dos años consecutivos. La iniciación, diferenciación y desarrollo de las yemas florales es un proceso continuo y relativamente corto. La floración del olivo se produce casi exclusivamente en brotes que se desarrollaron vegetativamente durante la primavera anterior a la floración, lo que quiere decir que las yemas que se siguen manteniendo latentes durante la primavera siguiente a su desarrollo suelen desarrollarse como brotes vegetativos y no como inflorescencias. De este modo, las yemas de los brotes desarrollados durante otoño tampoco suelen diferenciarse en inflorescencias (Lavee, 1996).

La diferenciación de las yemas florales se inicia a finales de invierno, pero la inducción se produce mucho antes, pocas semanas después de que se produzca la fecundación durante la temporada anterior. Durante las primeras semanas de junio ya se aprecia un cambio bioquímico en las yemas que las predestina a convertirse en yemas reproductoras durante la siguiente primavera (Rallo y Martín, 1991; Andreini et al., 2008). Las yemas se forman en las axilas de las hojas de los brotes en crecimiento. El crecimiento y desarrollo de éstas se completa en las semanas siguientes al inicio de su formación, desde que las yemas se localizan en el primer nudo apical hasta que se sitúan en el cuarto o quinto nudo debido al crecimiento del brote portador. A partir de este momento la morfología de la yema no se modifica hasta el comienzo de su brotación al año siguiente, es decir, la yema permanece latente. El destino de la yema, floral o vegetativo, depende probablemente de los estímulos que la yema posee durante su desarrollo en el verano. Se ha observado que las semillas del fruto recién formado juega un fuerte papel como inhibidor de la inducción floral sobre las yemas (Fernández-Escobar et al., 1992). Con la bajada de las temperaturas invernales, el árbol entra en un estado de reposo vegetativo y reproductor, denominado latencia. El proceso del desarrollo que conduce a la finalización de la latencia de las yemas está regulado principalmente por la temperatura (Galán et al., 2001a; Aguilera et al., 2013). Un periodo de bajas temperaturas, seguido por otro de temperaturas más elevadas, conduce a la brotación de las yemas. La latencia se puede separar en dos períodos: un periodo de "descanso" o "endolatencia", durante el cual las yemas están inactivas debido a las condiciones fisiológicas del árbol, y un periodo "quiescence" o "ecolatencia", como el período en el que las yemas permanecen inactivas debido a las condiciones ambientales desfavorables. Durante el periodo de "descanso" se precisa la acumulación de una determinada cantidad de frío para poder alcanzar el periodo "quiescente", durante el cual se precisa la acumulación de calor por parte de la planta para que se produzca el desarrollo de las yemas (De la Rosa et al., 2000).

La diferenciación morfológica entre yemas vegetativas y reproductoras se percibe visualmente sólo a finales del invierno o principios de la primavera, cuando se inicia el desarrollo activo de las yemas (De la Rosa et al., 2000; Mert et al., 2013). Sin embargo, diferencias bioquímicas entre yemas reproductoras y vegetativas pueden observarse durante otoño, o incluso durante verano (Andreini et al., 2008; Malik y Bradford, 2006; Pinney y Polito, 1990). Dentro de cada flor, los pétalos son lo primero en desarrollarse y poco después se diferencian los sépalos. El estambre se inicia unas tres semanas más tarde y el pistilo es el último órgano en desarrollarse pocos días después. Todo el proceso de diferenciación de la inflorescencia tarda unas 4-5 semanas. El posterior desarrollo de la inflorescencia debe considerarse un proceso de crecimiento de los órganos florales ya existentes (Barranco et al., 2008).

Durante la floración existe un periodo de polinización efectivo en el que se produce la mayor parte de la fecundación (Orlandi et al., 2005; Cuevas et al., 2009). Una vez fertilizado el óvulo, se desarrolla un fruto tipo drupa. Primero se produce una rápida división celular, el fruto puede apreciarse los 10-15 días posteriores a la fertilización. Posteriormente se produce el desarrollo del endocarpo a comienzos de la época estival (Rapoport et al., 2013). El desarrollo y crecimiento del fruto se desaceleran posteriormente, culminando el desarrollo del embrión y del endocarpo. A finales de julio se produce el engrosamiento de lo que será la pulpa con la biosíntesis de aceite, llegando el fruto a la maduración verde en septiembre. El envero comienza a continuación, y la maduración completa de la mayoría de los cultivares sólo se alcanza en el invierno (Shulman y Lavee, 1979).

En la Figura 10 se ha representado un cronograma modelo del desarrollo vegetativo y reproductor del olivo en condiciones climáticas estándar en la provincia de Córdoba (Barranco et al., 2008; Lavee, 1996).

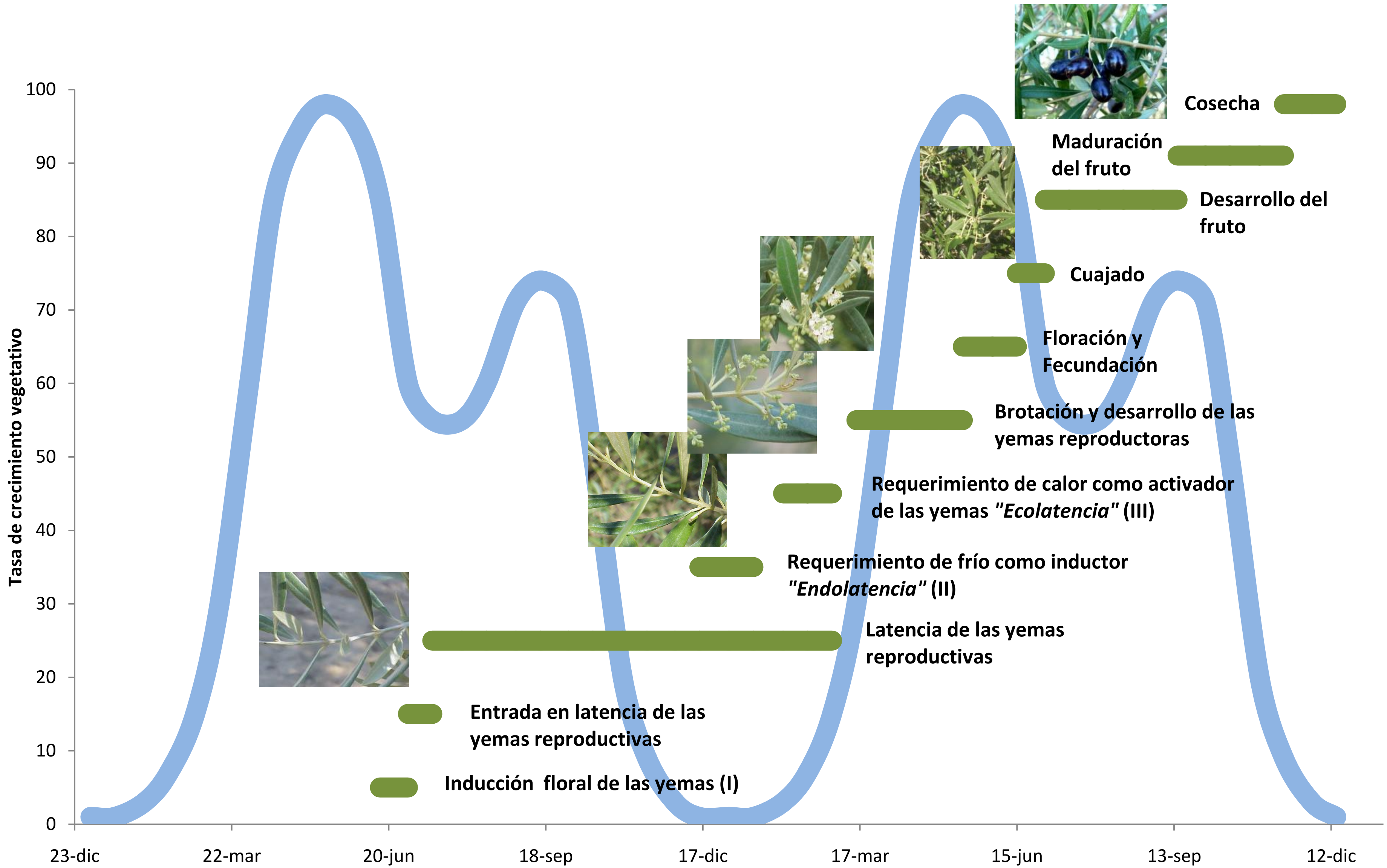

Figura 10. Cronograma modelo del desarrollo vegetativo (azul) y reproductor (verde) del olivo.

2.2.5.3 Vecería

El olivo, al igual que otros árboles frutales, sufre el fenómeno tradicionalmente conocido como “vecería”. Esta especie posee en condiciones ambientales estándar un patrón alternante en la intensidad de la floración, que deriva en una marcada alternancia de producción de fruto. Este patrón bianual en la intensidad de formación de órganos del ciclo reproductor en olivo se corresponde con un comportamiento inverso de crecimiento vegetativo, de modo que en años de “carga” de fruto el crecimiento vegetativo es menor que en años de “descarga” de fruto (Díaz-Fernández et al., 2004; Lavee, 2007).

La producción de fruto y las características fenológicas de esta especie son los principales responsables del comportamiento “vecero” debido a tres aspectos. En primer lugar, el fruto posee un mecanismo de control que influye sobre la inducción floral de las yemas (Fernández-Escobar et al., 1992). La carga de fruto induce la producción de ácido clorogénico por parte de las hojas, lo que inhibe la inducción reproductora de las yemas (Lavee, 1988; Ryan et al., 2003). En segundo lugar, el crecimiento de los órganos vegetativos y de los órganos reproductores se produce al mismo tiempo, este solapamiento fenológico es responsable de la existencia de una gran competencia entre ambos órganos por los foto-asimilados. El fruto del olivo se desarrolla en el brote vegetativo de un año formado durante la estación anterior. Por tanto, la longitud de ese brote es determinante del potencial de fructificación durante la estación siguiente. (Cuevas et al., 1994). En tercer lugar, también se ha detectado que la viabilidad de las flores en las inflorescencias y el porcentaje de cuajado serán menores tras un año “de carga” (Lavee, 2007; Troncoso de Arce et al., 2012).

Es remarcable el hecho que dentro de una determinada población pueden coexistir árboles que presenten “vecería” asincrónica entre ellos, e incluso dentro de un mismo árbol se pueden encontrar ramas en contra alternancia. Además, como ya se ha comentado, este ciclo alternante está influenciado por las condiciones meteorológicas que pueden romperlo durante varios años. Por otro lado, un evento climático extremo puede servir como espoleta de salida para iniciar una sincronía en la “vecería” de los árboles de la zona (Galán et al., 2008). Recientemente se están aplicando técnicas de cultivo con el objetivo de disminuir este comportamiento: irrigación, recolección temprana o el “aclareo artificial de fruto” (Dag et al., 2010).

2.3 Fenología del olivo.

La Fenología se define como la ciencia que comprende el estudio y la observación de los estadios de desarrollo reproductivo y vegetativos de plantas y animales en relación con los parámetros ambientales (Schwartz, 1999). Aunque la primera vez que se utilizó el concepto de Fenología fue en 1853 por el botánico Carles Morren, el término fue definido por primera vez en 1985 por Font Quer, como el estudio de los aspectos que se suceden en la vegetación de una especie, dependiendo de su propia idiosincrasia y del ciclo de dinamismo del medio, sobre todo del ciclo climático.

Si bien existen registros escritos sobre observaciones fenológicas que datan de miles de años, por ejemplo sobre la floración del cerezo en la corte real de Kioto hacia el año 705 a.C., las primeras observaciones sistemáticas continuadas de eventos fenológicos no se registran hasta el siglo XVIII (Menzel, 2002). La Fenología posee múltiples aplicaciones bien conocidas para Ciencias Naturales, Agronomía, Ciencias Forestales, salud humana, logística y transportes o turismo entre otros campos (Schwartz, 2003); pero recientemente se ha incrementado considerablemente el interés que suscita, principalmente debido a que el cambio del clima que se viene observando de manera generalizada durante las últimas décadas está produciendo una clara respuesta en plantas y animales. Algunos trabajos demuestran que la expresión fenológica es un excelente bioindicador de los efectos del cambio climático (García-Mozo et al., 2010b; Gordo y Sanz, 2010; Menzel, 2000); pero además, recientemente, los datos fenológicos han tomado un valor añadido debido a otros de sus usos como calibradores y evaluadores de la información de satélite NDVI o por su importancia como variable ecológica (Tucker et al., 2001; Chen et al., 2001; Bradley et al., 2011).

La obtención de datos fenológicos requiere el seguimiento de una metodología determinada. De manera generalizada todos los métodos de seguimiento fenológico se basan en la asignación de una clave a cada estado de desarrollo fenológico, que varía a medida que avanza el desarrollo. Durante la presente tesis se han seguido las claves fenológicas estandarizadas de la BBCH para la fenología floral olivo, al tratarse de la escala fenológica estandarizada más aceptada a nivel mundial (Figura 11). La escala BBCH es un sistema para una codificación uniforme de identificación fenológica de estadios de crecimiento para especies de angiospermas desarrollado por Zadoks et al. (1974). Siguiendo el código BBCH, se ha descrito de manera específica el desarrollo fenológico del olivo en otras publicaciones (Sanz-Cortés et al., 2002). Cada etapa en esta escala fenológica representa un estado secuencial en la evolución de las yemas que dan lugar a brotes, por un lado, y a flores hasta la formación de frutos por otro.

El estudio de la fenología en cualquier planta tiene especial interés por su relación con el clima en general, y el microclima en particular, en el que se desarrolla la planta, actuando en este caso como un indicador biológico del mismo. También desde el punto de vista agronómico sirve de guía en las diversas actuaciones que se realizan sobre una planta cultivada, como pueden ser los tratamientos fitosanitarios, las podas, etc. Esta variable es muy útil para conocer la adaptación de una planta a condiciones distintas de las originales.

La Fenología secuencial subdivide el desarrollo de un taxón biológico en secciones identificables a lo largo del tiempo, y estas subdivisiones son conocidas con el nombre de fenofases, cuya sucesión en el tiempo se utiliza para monitorizar diversos aspectos del desarrollo estacional en la vegetación (Lieth, 1997). A través de las observaciones fenológicas efectuadas directamente sobre el individuo, se hace posible la cuantificación de la evolución de la fenología de una especie en forma de tendencia fenológica.

El estudio de la fenología floral del olivo se extiende desde el inicio de la brotación, a finales de invierno, hasta el cuajado del fruto, a principios de verano, analizando todas las etapas de desarrollo floral. El tiempo transcurrido desde la formación de la primera flor hasta la última no suele exceder de 1-2 semanas durante el periodo de floración (Vázquez, 2000). Una vez iniciado este proceso, la yema continúa su desarrollo ininterrumpidamente hasta que se abre y emerge la inflorescencia.

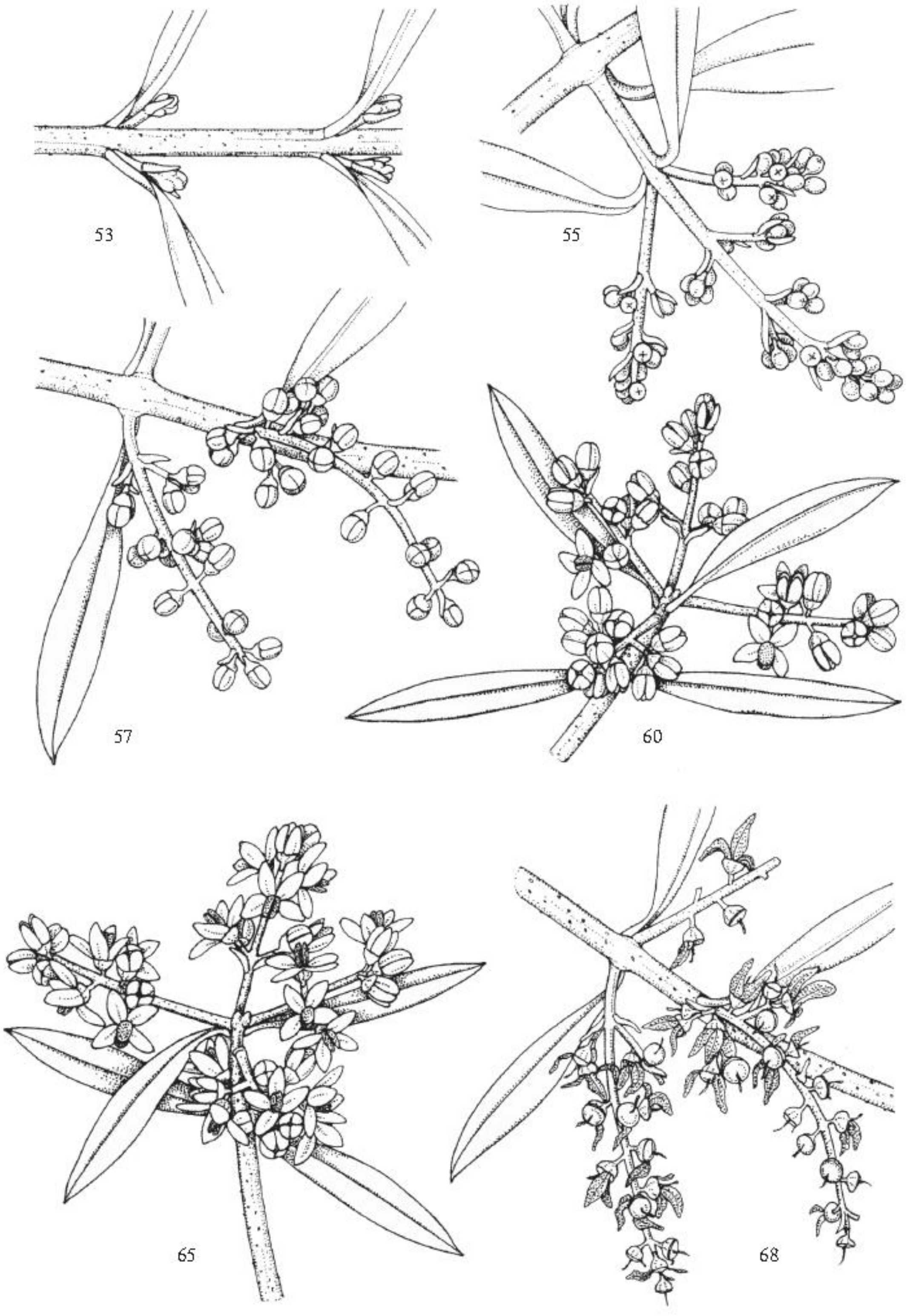

Figura 11. Algunas fases de la emergencia y desarrollo de la floración del olivo de acuerdo a la escala fenológica estandarizada de la BBCH: 53, desarrollo temprano de los botones florales; 55, las inflorescencias alcanzan su tamaño final; 57, aparición de la corola verde; 60, presencia de flores abiertas; 65, plena floración; 68, crecimiento ovárico y caída de pétalos. (Sanz-Cortés et al., 2002)

El crecimiento inicial de la inflorescencia es uniforme y simultáneo para todas sus partes (Figura 12a, 12b). Una vez que la inflorescencia está formada y alcanza una longitud de unos 2 cm, el raquis comienza a alargarse rápidamente (Figura 12c). Cuando la inflorescencia llega a unos 2/3 de su longitud total, las flores individuales comienzan a crecer. El tamaño final de la inflorescencia y de las flores se alcanza justamente antes de la floración, entre mediados de abril y mediados de mayo, según las condiciones ambientales y el cultivar (Figura 12d). El color de la inflorescencia en crecimiento es verde y la clorofila desaparece de los pétalos sólo un poco antes de la apertura de la flor. En esta fase, los pétalos de la flor del olivo de la mayoría de los cultivares se vuelven blancos justo antes de la floración (Figura12e, 12f) (Vázquez, 2000).

El número total de flores de las inflorescencias, su distribución en el raquis y la longitud de la inflorescencia están determinas genéticamente y, por tanto, son específicos de cada cultivar. No obstante los tres caracteres citados presentan diferencias dentro del mismo árbol. Las inflorescencias de los extremos proximal y distal del brote suelen ser más pequeñas y en el extremo proximal también desarrollan menos flores. El tamaño de las inflorescencias y el número de flores varían también de un año a otro, de acuerdo con el estado fisiológico del árbol y con las condiciones meteorológicas. Una vez finalizada la floración y fecundación, los pétalos se tornan marrones (Figura 12g), terminan cayéndose y se inicia el crecimiento ovárico y el proceso de cuajado de frutos (Figura 12h) (Lavee, 1996). De todos los factores que pueden afectar al desarrollo de las estructuras reproductoras, la temperatura ejerce el mayor efecto en el mismo, especialmente en la formación de las estructuras florales (Chuine et al., 1998). La diferencia de temperatura en las diferentes localizaciones geográficas es considerada la principal fuente de variación geográfica en la fenología (Wielgolaski, 2003; Milla et al., 2009; Aguilera, 2012; Jochner et al., 2012).

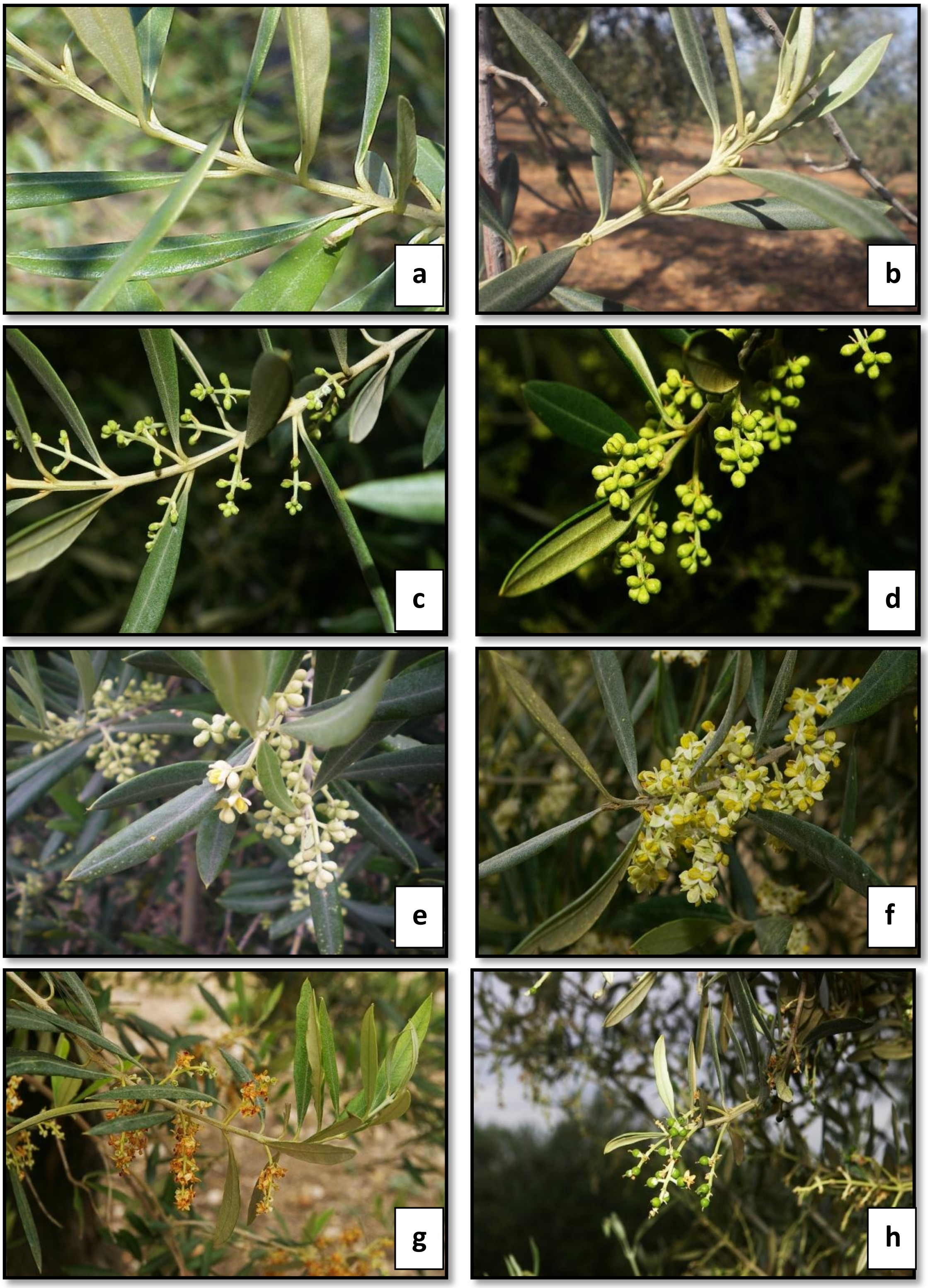

Figura 12. Desarrollo fenológico de la floración del olivo. Según la escala fenológica estandarizada de la BBCH, las figuras se corresponden con las siguientes fenofases: a-50, yema en reposo; b-51, salida de la dormancia; c-54, formación del botón floral; d-57, diferenciación de la corola; e-61, inicio de la floración (<15% de flores abiertas); f-65, plena floración (>15% de flores abiertas); g-67, fin de la floración (<15% de flores abiertas); h-68, crecimiento ovárico y caída de pétalos.

2.4 Aerobiología del olivo.

La Aerobiología se define como la ciencia que estudia los microorganismos y el material de origen biótico que se transportan y/o dispersan de forma pasiva a través del aire.

Los estudios aerobiológicos poseen distintas aplicaciones directas, las principales se encuadran en el campo de la Medicina (atopía y asma, infecciones fúngicas, ciertos contaminantes aerotransportados) (D'Amato et al., 2007), en la Agricultura (aparición de plagas, viabilidad de cultivos, predicción de cosecha) (Frenguelli, 1998; García-Mozo, 2011), en Fenología (Damialis et al., 2007;Rodríguez-Rajo et al., 2009), en el campo de la Ecología (estudios sobre cambio climático, relaciones ecológicas, cambios en los ecosistemas) (Ziello et al., 2012; García-Mozo et al., 2010), y sobre el biodeterioro (estudios sobre biodeterioro fúngico) (Ruga et al., 2008), entre otras. Según Frenguelli (2013), los estudios aerobiológicos se están enfocando en: estandarización del método aerobiológico, análisis del polen y esporas, análisis de alérgenos, análisis de partículas aerovagantes y de hongos fitopatógenos, estudios de cambio climático y de fenología y estudios sobre modelización. Y según Comtois (2011), teniendo en cuenta los resultados publicados durante las últimas dos décadas, los futuros retos de la Aerobiología se enmarcan en los campos de la Aerobiología teórica, el desarrollo de nuevos sistemas de monitorizaje y del desarrollo de nuevos sistemas de información aerobiológica. La mejor cuantificación del potencial alergénico de los conteos polínicos también es otro de los nuevos retos de la Aerobiología (Cechi, 2013). No obstante, aún hoy, quedan por resolver cuestiones básicas en relación al empleo de un método estandarizado (Mandrioli y Ariatti, 2001).

El olivo ha sido una de las especies más estudiadas a nivel aerobiológico en el Área Mediterránea, ya que, debido a su alta producción de polen y a la gran superficie dedicada a este cultivo, las concentraciones de este tipo polínico son muy importantes. Además, es una de las principales causas de alergia entre la población mediterránea (D'Amato et al., 2007; Barber et al., 2008). El gran interés socio-económico de esta planta también ha propiciado el estudio del polen aerovagante de olivo. Los trabajos científicos, con un enfoque aerobiológico, más relevantes se agrupan en aquellos con una finalidad descriptiva (i.e. Díaz de la Guardia et al., 1999; Ribeiro et al., 2005), aplicación fenológica (i.e. Melo-Abreu et al., 2004; Aguilera y Ruiz-Valenzuela, 2009), alérgenos (i.e. De Linares et al., 2007; Galán et al., 2013), viabilidad polínica (i.e. Aguilera y Ruiz-Valenzuela, 2013), predicciones de la fecha de floración (i.e. Ribeiro et al., 2006a; Avolio et al., 2008; Orlandi et al., 2010a), predicciones de intensidad de la floración a largo plazo (i.e. Galán et al., 2001b; Ribeiro et al., 2007; Oteros et al., 2013a, 2013b), predicciones de polen aerovagante a corto plazo (i.e. Vázquez et al., 2003; Díaz de la Guardia et al., 2003), predicciones de cosecha (i.e. García-Mozo et al., 2008; Galán et al., 2008; Ribeiro et al., 2008; Orlandi et al., 2010b) y análisis de retro-trayectorias (i.e. Hernández-Ceballos et al., 2010).

2.4.1 El polen de olivo y sus aeroalérgenos.

El olivo es una de las principales causas de alergia respiratoria en los países del mediterráneo, donde su cultivo se extiende ampliamente (D'Amato et al., 2007). España, el sur de Italia, Grecia y Turquía representan las áreas donde los casos de sensibilidad al olivo han sido más importantes (D'Amato y Lobefalo, 1989; Gioulekas et al., 2004; Kirmaz et al., 2005; Florido et al., 2009). Dentro de España, la provincia de Córdoba es una de las que concentra el mayor porcentaje de sensibilización al alérgeno mayoritario Ole1, junto a otras provincias del sur de la Península Ibérica como Jaén, Granada o Málaga (Barber et al., 2008). Cambios en el sistema de cultivos, en el ambiente y en las diferentes variedades cultivadas están produciendo el incremento del porcentaje de sensibilización (Liccardi et al., 1996; Conde-Hernández et al., 2002). Se ha demostrado que su alergenicidad es dependiente del cultivar (Soleimani et al., 2013). En la actualidad se han descrito 12 alérgenos en los granos de polen de olivo y, aunque no es muy frecuente que sus productos derivados produzcan alergia, también se ha descrito un alérgeno en su fruto (Esteve et al., 2012). El polen de olivo puede, por otro lado, causar reactividad cruzada con el de otras especies que intensifiquen los síntomas de alergia (Lombardero et al., 2002). El cuadro clínico de la polinosis al polen de olivo se caracteriza por rinoconjuntivitis y por asma bronquial (Liccardi et al., 1996). Aunque la cantidad de alérgenos que se detectan en el aire suele estar relacionada con las concentraciones de polen aerovagante de olivo, sin embargo, esta relación no está siempre definida (Fernández-González et al., 2010; Galán et al., 2013).

2.4.2 El método aerobiológico

El parámetro aerobiológico considerado en este estudio es el contenido de polen de olivo en el aire. El captador volumétrico tipo Hirst (Hirst, 1952) es el método propuesto por la *European Aeroallergen Network* (Jäger, 1995), el cual permite conocer las oscilaciones horarias y diarias de las partícula biológicas (Figura 13a,b). Estos captadores permiten obtener datos homologables, independientemente de las características biogeográficas y bioclimáticas de la zona en la que se realice el muestreo (Mandrioli et al., 1998). Para la obtención de los datos aerobiológicos se han seguido las recomendaciones que se detallan en el *Manual de Calidad y Gestión de la REA* (Galán et al., 2007)

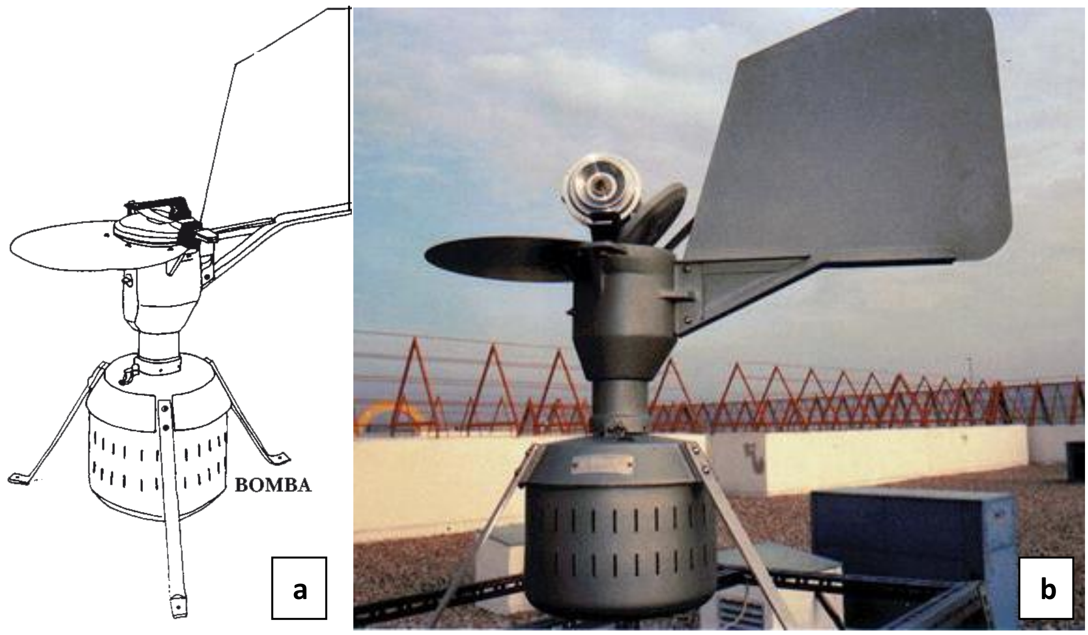

Figura 13. a. Captador volumétrico tipo Hirst; b. Captador volumétrico tipo Hirst localizado en el campus de Rabanales, Córdoba, sobre torre de elevación.

2.5 Manejo estadístico de los datos aerobiológicos y fenológicos

La obtención de resultados fiables en estudios aerobiológicos y fenológicos depende en gran medida del tamaño de las bases de datos analizadas, que determinan el tamaño muestral. Las matemáticas y la estadística se convierten en herramientas imprescindibles para poder extraer conclusiones científicas en cualquier área del conocimiento, pero en los estudios aerobiológicos son además un medio esencial para tratar, ordenar y extraer la información útil de estas bases de datos. Uno de los objetivos de la tesis es la modelización de los principales aspectos del ciclo reproductor del olivo, por ello se ha realizado un estudio detallado del tratamiento estadístico más adecuado en cada caso.

2.5.1 Tratamiento y manejo de datos

El primer tratamiento matemático que sufren los datos que obtenemos del muestreo directo del aire es la transformación del número bruto de partículas en datos de concentración de polen por volumen. Para ello es necesario obtener un factor de transformación específico en cada caso. Para realizar esta transformación se tiene en cuenta el volumen de succión del Hirst (10 l/min), el diámetro de campo de visión del microscopio y el área de la muestra analizada (Galán et al., 2007). Es importante analizar la calidad de los datos ya que éstos suelen estar sometidos a diversas fuentes de error (error instrumental, error de protocolo, bias…), por lo que es necesario que durante todo el proceso de obtención de datos se cumplan las medidas de calidad oportunas (Oteros et al., 2013c). Los “outliers” y los “missing data” también deben ser analizado ya que pueden modificar el resultado final del estudio. Otro aspecto a tener en cuenta es la precisión máxima del captador y la finalidad del estudio antes de determinar la estructura temporal de los datos (Mandrioli et al., 1998).

Los datos fenológicos, por otro lado, son tomados como fechas en las que se producen los distintos eventos fenomorfológicos. En el pre-tratado de datos fenológicos, es muy frecuente el empleo de la interpolación como técnica para calcular la fecha en la que se han producido los principales eventos o para el cálculo de amplitudes fenológicas.

2.5.2 Análisis estadístico

Tanto en Aerobiología como en Fenología, antes de utilizar cualquier técnica estadística es imprescindible tener un objetivo claro de la finalidad del trabajo. Dependiendo ésta y de las características de los datos podrán emplearse distintas técnicas. A lo largo de la tesis se han empleado diferentes técnicas que podrían ser agrupadas en cuatro grupos atendiendo a la finalidad de las mismas:

a. Comparación de medias

Uno de los análisis estadísticos preliminares llevados a cabo en la presente tesis es la comparación de medias. Para este fin existen diferentes técnicas. Antes de aplicar cualquiera de ellas se debe se debe tener en cuenta si se pretenden comparar dos grupos de datos o más de dos grupos, si los datos se ajustan a una distribución normal o no y si las mediciones entre los grupos son independientes o mantienen relación de dependencia (por ejemplo cuando se realizan medidas repetidas en el tiempo o los datos se encuentran pareados). La normalidad se puede testar observando un "Q-Q plot" o mediante las pruebas de "Kolmogorov-Smirnov" o de "Shapiro-Wilk", entre otras. Teniendo en cuenta estas premisas se puede seleccionar alguna técnica de la tabla 1, como ejemplos de métodos de comparación de medias. Una vez detectadas diferencias entre varios grupos (más de dos) se pueden utilizar las denominadas técnicas "Post-Hoc", como por ejemplo el "Tukey test" o el "Duncan Test", para conocer exactamente en que grupos se presentan las diferencias significativas; la elección de la técnica post-hoc depende de la finalidad del estudio y de la flexibilidad requerida en el resultado final (Carmer y Walker, 1982). La comparación de medias se aplica usualmente en estudios aerobiológicos y fenológicos (Alcázar et al., 1999, 2009; Rodríguez-Rajo et al., 2010; Velasco-Jiménez et al., 2013; Cotos-Yáñez et al., 2013). En el capítulo III de la presente tesis se ha empleado la técnica "Sign test" para testar las diferencias entre dos grupos de datos fenológicos que mantienen una relación de dependencia temporal Oteros et al., (2013d).

Comparación de medias				
	2 medias		Más de 2 medias	
	Paramétrico	No Paramétrico	Paramétrico	No Paramétrico
Datos independientes	T-Student	U Mann-Whitney	ANOVA	H Kruskall Wallis
Datos dependientes	T-Student para datos pareados	Wilcoxon test Sign test	General Linear Mixed Models	Generalized Linear Mixed Models

Tabla 1. Técnicas estadísticas de comparación de medias.

b. Análisis de series temporales

Muchos estudios de Aerobiología y de Fenología poseen como objetivo el análisis de la tendencia en series temporales, en estos casos se suele utilizar la "Regresión Lineal" (i.e. García-Mozo et al., 2010b), además del empleo de las técnicas no paramétricas de "Sen" y de "Mann-Kendall" para analizar la tendencia en series temporales de fechas de floración (Orlandi et al., 2010c). Aunque ningún capítulo de la presente tesis posee como objetivo el análisis de series temporales, se anexionan otras contribuciones científicas del doctorando en las que se han utilizado otros métodos estadísticos con esta finalidad, tales como la "Regresión lineal", modelos "ARIMA" o la "Descomposición de series temporales por el método de Loess" (García-Mozo et al., 2014).

c. Técnicas de agrupación

En caso de que el objetivo del estudio aerobiológico sea la agrupación de datos en diferentes grupos se suelen emplear métodos como el "Análisis de componentes principales (PCA)", árboles de decisión o diferentes métodos "clustering" (i.e. Voukantsis et al., 2013; Grinn-Gofrón y Bosiacka, 2012; Grishkan et al., 2012). En el capítulo II de la presente tesis se emplean el método de "conglomeración jerárquica" para conocer el número "K" exacto de grupos naturales de años en base a sus características biometeorológicas, y el método de "conglomeración K-means" para agrupar dentro de los "K" grupos a los años en base a sus características.

d. Otras técnicas de modelización

Cuando se trata de modelizar un comportamiento natural, como son los niveles de polen que se detectan en el aire o la fecha o duración de una fase fenológica, se trata de describir matemáticamente procesos muy complejos, que pueden depender a su vez de múltiples subprocesos implicados en la Aerobiología y en la Fenología, como el proceso de emisión, de transporte, de deposición, de resuspensión o de finalización de la dormancia. De este modo se han desarrollado distintos métodos útiles para describir relaciones entre variables dependientes e independientes. Cuando se trata de modelizar procesos complejos, como es la dinámica del polen aerovagante, se recomienda dividir el problema para modelar procesos más simples y fáciles de explicar, de este modo es frecuente dividir la estación polínica en varios periodos fenológicos, como son el periodo pre-pico y el post-pico, en los que pueden estar interviniendo fenómenos diferentes.

Normalmente, cuando se clasifican las técnicas de modelización se suele hacer atendiendo a sus características matemáticas, como ejemplo: métodos univariantes o multivariantes; a el modo de relacionar la variables, como ejemplo, modelos de caja negra o modelos de caja blanca, a si son o no son modelos autorregresivos; a si se analizan factores aleatorio o factores fijos; a si la variable dependiente posee una distribución normal o no. En este estudio se han clasificado las técnicas dependiendo de la naturaleza de la variable dependiente:

- <u>Variable dependiente cualitativa.</u> Se trata de modelos de clasificación como la “Regresión logística” o el análisis “SIMCA”. En el capítulos II de la presente tesis se han comparado varios métodos de clasificación: “Redes neuronales artificiales”, “Árboles de decisión” y “Análisis discriminante”.

- <u>Variable dependiente cuantitativa.</u> Éste es el tipo de modelos más frecuente en Aerobiología y en Fenología. Los modelos más frecuentes en ambas disciplinas se suelen realizar para modelizar y predecir el comportamiento a corto plazo de las series diarias de polen (i.e. Galán et al., 2000; Grinn-Gofrón y Strzelczak, 2008), para modelizar el comportamiento a largo plazo de intensidad de la floración en diversas especies (i.e. Orlandi et al., 2010c; García-Mozo et al., 2012) o para modelizar el comportamiento fenológico (i.e. Rodríguez-Rajo et al., 2011; Pauling et al., 2012). Las técnicas estadísticas más empleadas en este tipo de casos son la correlación simple "Pearson" y "Spearman" y la "Regresión Lineal Múltiple", aunque existe un gran abanico de técnicas estadísticas disponibles para realizar este tipo de análisis. Durante la ejecución de la tesis se han empleado "Correlación" y "Regresión lineal múltiple" en el capítulo I, en los capítulos II, III y IV se ha empleado una potente técnica multivariante para modelizar este tipo de relaciones, el análisis de "Regresión por el método de los mínimos cuadrados parciales" (Oteros et al., 2013a, 2013b, 2013d, 2013e). En el capítulo III también se han utilizado las técnicas de "Regresión lineal múltiple" y de "Correlación" para modelizar el comportamiento fenológico del olivo.

- Variable dependiente georrepresentada, <u>Modelos Geoespaciales</u>. La mejora tecnológica en hardware y software que se ha venido produciendo durante los últimos años han permitido que este tipo de modelos sean cada vez más utilizado en Aerobiología y Fenología (i.e. Alba et al., 2006), empleando técnicas de geoestadística como el "Kriging" o el "Co-Kriging". En la introducción de la presente tesis se hace uso de los "Modelos de máxima entropía (MAXENT)" para calcular el área bioclimática potencial del olivo.

En el capítulo II de la presente tesis se desarrolla un novedoso método que ha sido llamado "Three-step method", basado en una combinación de algunas de las técnicas descritas anteriormente (Oteros et al., 2013b). Primero una Agrupación, mediante una técnica "clustering jerárquico y clustering k-means", seguido de una segunda Clasificación, mediante "Redes neuronales artificiales" y una tercera Modelización, mediante "Regresión por el método de los mínimos cuadrados parciales". Mediante esta metodología se pueden realizar predicciones del índice polínico con varios meses de antelación y profundizar más efectivamente en la bioclimatología de especies como el olivo.

3. OBJETIVOS

3. Objetivos

El objetivo principal de la presente tesis ha sido el de caracterizar los requerimientos bioclimáticos del olivo (*Olea europaea* L.) durante su ciclo reproductor en la provincia de Córdoba (Andalucía, España). Para ello se proponen modelos que también pueden ser aplicados para generar predicciones precisas de la intensidad de la floración (capítulos I y II), del periodo de la floración (Capítulo III) o de la cosecha de aceitunas (Capítulo IV). Éste objetivo principal se ha abordado a lo largo de toda la tesis, planteándose objetivos más específicos en cada uno de los diferentes capítulos que se detallan a continuación:

Capítulo I

Biometeorological and autoregressive indices for predicting olive pollen intensity .

1. Analizar las condiciones ambientales que influyen sobre la intensidad de la floración, en forma de Índice Polínico, en la provincia de Córdoba.

2. Generar un modelo que sea capaz de realizar predicciones precisas del Índice Polínico en la provincia de Córdoba.

3. Desarrollar una técnica estadística que permita tener en cuenta el efecto no lineal de los eventos extremos del clima mediterráneo sobre la intensidad de la floración, mediante el desarrollo y optimización de índices biometeorológicos.

4. Analizar el comportamiento cíclico de la serie temporal de Índices Polínicos del olivo y generar un índice que recoja esta información, el Índice de Ciclicidad, que pueda ser utilizado para realizar predicciones.

5. Comparar la eficacia del empleo de índices en los modelos de predicción de la intensidad de la floración.

Capítulo II

Year clustering analysis for modelling olive flowering phenology.

6. Analizar las condiciones ambientales que influyen sobre la intensidad de la floración, mediante un enfoque "*clustering*", lo que permitirá analizar el efecto de las mismas condiciones meteorológicas ante diferentes marcos ambientales.

7. Generar un modelo que sea capaz de realizar predicciones precisas del Índice Polínico en la provincia de Córdoba mediante un enfoque "*clustering*".

8. Generar una metodología estadística novedosa denominada "*three-step method*", que consiste en realizar una agrupación en primer lugar, posteriormente una clasificación y finalmente una modelización.

9. Comparar la eficacia de diferentes métodos de clasificación (árboles de decisión, análisis discriminante y redes neuronales artificiales) en los modelos de predicción del Índice Polínico.

Capítulo III

Modelling olive phenological response to weather and topography.

10. Conocer cuál es la frecuencia óptima para realizar un monitorizaje de la fenología floral durante el periodo reproductor del olivo.

11. Analizar cuáles son las principales causas topográficas que determinan el comportamiento fenológico del olivo en la provincia de Córdoba.

12. Analizar los efectos de la meteorología sobre el comportamiento fenológico del olivo en la provincia de Córdoba.

13. Generar modelos de previsión eficaces sobre el inicio del desarrollo morfológico de las yemas florales, el inicio de la floración y de la fecha de máxima floración.

Capítulo IV

Better prediction of Mediterranean olive production using pollen-based models.

14. Generar modelos integrados a gran escala que resuman las condiciones ambientales que afectan a la producción de fruto de olivo en la cuenca del Mediterráneo

15. Analizar las variaciones en la producción de cosecha en tres de los principales países productores (España, Italia y Túnez).

16. Generar modelos de ámbito regional que sean útiles para entender los requerimientos ambientales más específicos del olivar en cada zona de estudio.

17. Generar modelos que se puedan emplear para realizar predicciones precoces de cosecha de aceitunas en diferentes países mediterráneos.

4. CAPÍTULO I

Biometeorological and autoregressive indices for predicting olive pollen intensity

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ORIGINAL PAPER

Biometeorological and autoregressive indices for predicting olive pollen intensity

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Abstract This paper reports on modelling to predict airborne olive pollen season severity, expressed as a pollen index (PI), in Córdoba province (southern Spain) several weeks prior to the pollen season start. Using a 29-year database (1982–2010), a multivariate regression model based on five indices—the index-based model—was built to enhance the efficacy of prediction models. Four of the indices used were biometeorological indices: thermal index, pre-flowering hydric index, dormancy hydric index and summer index; the fifth was an autoregressive cyclicity index based on pollen data from previous years. The extreme weather events characteristic of the Mediterranean climate were also taken into account by applying different adjustment criteria. The results obtained with this model were compared with those yielded by a traditional meteorological-based model built using multivariate regression analysis of simple meteorological-related variables. The performance of the models (confidence intervals, significance levels and standard errors) was compared, and they were also validated using the bootstrap method. The index-based model built on biometeorological and cyclicity indices was found to perform better for olive pollen forecasting purposes than the traditional meteorological-based model.

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Introduction

The olive-tree (*Olea europaea L.*) is a characteristic Mediterranean species; olives and olive oil are amongst the oldest agricultural products in this part of the world. The region of Andalusia (southern Spain) boasts the world's largest area of olive-groves (1,511,687 ha in 2009), accounting for one-third of all Andalusian cropland. The region produces over 5 million tonnes of olives per year. Córdoba province is the second largest producer after Jaén, with a surface area of 343,812 ha and an annual output of 1,064,997 tonnes of olives (Andalusia Statistical Yearbook 2010). Most representative olive cultivars in Córdoba are Hojiblanca, Picudo and Picual.

Although the olive-tree was originally an insect-pollinated species, today—due partly to intensive crop practices—wind-pollination also plays a major role (Dominguez et al. 1993a). In anemophilous species, the pollen index (PI), i.e. the detected annual airborne pollen, is regarded as a good indicator for both flowering intensity and plant behaviour during certain phenological stages (Keynan et al. 1989; Aguilera and Ruiz Valenzuela 2009).

In view of the economic and environmental importance of the olive, there has been considerable research into flowering phenology and its aerobiological characteristics (Vázquez et al. 2003; Díaz de la Guardia et al. 2003, García-Mozo et al. 2006; Hernández-Ceballos et al. 2010). Olive pollen grains contain a number of different allergens that give rise to atopy and asthma in a large proportion of the population throughout the Mediterranean area (D'Amato et al. 1998; Barber et al. 2008). Since it covers such a large surface area in the Andalusia region, the olive-tree is the leading cause of allergies in this area (Domínguez et al. 1993b). The knowledge in advance of airborne pollen concentrations would be of undoubted value both for allergy specialists, allowing them to plan treatments, and for allergy-sufferers, who could better organize their activities.

Aerobiology and phenology have also aroused considerable interest in the agricultural sciences. In fact, olive PI has proved to be a good indicator for predicting harvests (Galán et al. 2004; García-Mozo et al. 2008; Orlandi et al. 2010a). Armed with detailed knowledge of this index together with factors influencing it, farmers can minimise the environmental impact of crop treatments and have a clear idea of the size of the upcoming crop, thus enabling more efficient resource management for crop distribution and price marketing. Moreover, knowledge regarding the influence of weather-related parameters on pollen production is essential to any decision regarding the introduction of certain olive varieties in new areas.

The bioclimatic requirements for flowering in the olive vary as a function of the tree's phenological status (Fornaciari et al. 1998; Galán et al. 2001a). In late autumn and early winter, olive-trees undergo a dormancy period, when growth and development are temporarily suspended (Melo-Abreu et al. 2004). Afterward, a situation of stress due to low temperatures is required to emerge from dormancy (chilling period). Once the threshold temperature is reached, the plant accumulates heat units until flowering starts, (Galán et al. 2001a). Therefore, knowledge about the influence of previous biometeorological factors influencing pollen production would help researchers to have more accurate predictions regarding the potential effect of climate change on olive flowering development (Galán et al. 2005; García-Mozo et al. 2010a).

Most research into long-term airborne olive-pollen forecasting has focussed on predicting other phenological stages of the olive breeding cycle, especially the onset of flowering (Frenguelli et al. 1989; Alcalá and Barranco 1992; Orlandi et al. 2010b). However, a number of studies have sought to forecast the olive PI in the Mediterranean area by constructing regression models and using simple meteorological-related variables, concurring that the variables that affect pollen production are moderate temperatures and abundant rainfall during March and April, among other less important variables (Galán et al. 2001b; Fornaciari et al 1997; Ribeiro et al. 2006).

The main objectives of this work were: (1) to propose an accurate model to predict olive PI in Córdoba province. With this purpose in mind, we tested the accuracy of a meteorological-based model constructed using simple meteorological-related variables versus an index-based model based on biometeorological indices and an autoregressive index; (2) to include the influence of Mediterranean extreme climate events on olive pollen production in the proposed Index-based Model by applying different adjustment criteria; and finally (3) to consider the cyclical component of PI series by the development of a cyclicity index.

This paper reports on the comparison and validation of the two models in order to select the best model to obtain accurate forecasting by taking into account all the potential facts influencing PI variation in Córdoba province.

Materials and methods

Study area and pollen data

The city of Córdoba is situated in the south-west of the Iberian Peninsula (37°50′N, 4°45′O), at a height of 123 m above sea level. It has a Mediterranean climate with some continental features. The annual average temperature is 17.8 °C and the mean annual rainfall is 621 mm. Pollen counts and weather data for the last 29 years (1982–2010) were used for this study. Weather data, provided by the Spanish State Meteorology Agency (AEMET), were based on readings taken at Córdoba Airport, located around 5 km south of the pollen sampler site. Aerobiological data were collected using a Hirst-type volumetric spore trap (Hirst 1952) placed on the roof of the Educational Sciences Faculty, at 15 m above ground level. Pollen counts were obtained using a standard protocol published by the *Spanish Aerobiology Network* (REA) (Galán et al. 2007).

Building of biometeorological indices

The general steps of the study were to build, validate and compare the performance of the created models for forecasting olive PI with both the meteorological-based model and the index-based model.

The bioclimatic indices required for constructing the index-based model were generated during this study. Under a test correlation among the different meteorological-related variables and PI, those exerting most influence on flowering severity in the province of Córdoba were selected. Table 1 shows only those variables that have a higher correlation coefficient, i.e. the variables selected to build the biometeorological indices (Table 1). Two criteria were followed to combine the variables in building the indexes: regarding the first criterion, a discrimination was made between both heat (temperature) and water-related (rainfall) variables, while for the second criterion a discrimination was made between different seasons at which the variables can act in different manners (summer, autumn, winter and early spring). The combination of the variables selected previously to build indices was performed using multiplication or division, to use all the variables selected. Depending on the correlation observed, variables can appear in the formulas of the indices as a factor (if they show a positive correlation with PI) or splitter (given a negative correlation with PI). Considering the first criterion, in the construction of the indices, we have not used variables of thermal nature and hydric nature at the same time; for the second criterion, we did not employ variables acting on non-consecutive periods of time. For example, the March temperature cannot be combined with the August temperature for failing to meet the second criterion, nor combined with rainfall in March for failing to meet the first criterion; however, March temperatures can be combined with April temperatures if this variable were to be selected in the correlation test. The indices have been constructed specifically for the study area so should not be exported to other areas; however, the method per se can be exported to other areas. The variables that comprise each index are described in the Discussion and the results are consistent with those obtained in previous studies. The four biometeorological indices were defined as follows: thermal index (TI), pre-flowering hydric index (PFHI), dormancy hydric index (DHI) and summer index (SI).

Thermal index (TI): minimum March temperatures (minTM) and March temperature range (RanTM) were included in the TI as follows:

$$TI_0 = (minTM)/(RanTM)$$

Pre-flowering hydric index (PFHI): the water conditions most affecting reproductive development in early spring; sum of total rainfall in February (RfF) and total rainfall in March (RfM) were included in the PFHI.

$$PFHI = (RfF) + (RfM)$$

Dormancy hydric index (DHI): Cumulative rainfall from September to January (RfS↔J), and mean sunlight hours from September to January (HSunS↔J), as an indicator of water availability at the end of dormancy, were included in the DHI as follows:

$$DHI_0 = (RfS \leftrightarrow J)/(HSunS \leftrightarrow J)$$

Summer index (SI): minimum temperatures from June to September (minTJn↔S) and mean sunlight hours from June to August (HSunJn↔A) were included in SI as the most influential summer conditions over the summer index.

$$SI = (minTJn \leftrightarrow S) \times (HSunJn \leftrightarrow A)$$

Optimisation of biometeorological indices

Moreover we took into account that flowering can also be affected by certain weather conditions occurring prior to and during the pollen season. These conditions are generally the result of extreme climate events, the impact of which needs to be borne in mind. An attempt to include extreme meteorological events in the proposed index-based model was made by applying different adjustment criteria to the defined biometeorological indices. A new method based on extreme weather events has been also developed. This new method aims to optimize the RMSE between typified biometeorological indices and typified PI by adjustment criteria that awards “bonuses” or “penalties” to the biometeorological indices.

Identifying extreme meteorological events and adjustments criteria

Extreme meteorological events affecting flowering intensity were identified by applied the following criteria:

(1) Since the PI and the biometeorological index present different units of measurement, they were typified to avoid potential errors: $Z = \left(X_i - \overline{X}\right)/\sigma$
(2) To identify extreme events not hitherto reflected in earlier correlation analyses, were first searched years having extreme meteorological events with potential influence on olive flowering. Those years were identified as years in which the difference between typical PI and the biometeorological index >1.
(3) The search for extreme meteorological events was focussed on finding meteorological outliers in previously identified years. Such meteorological outliers are defined as a meteorological variable with values far away from their normal range as follows:
   – Values over the upper meteorological value limit, considering the upper limit (UL) as:

     $$UL = Q_3 + 2 \times (Q_3 - Q_1)$$

   – Values under the lower meteorological values limit, considering the lower limit (LL) as:

     $$LL = Q_1 - 2 \times (Q_3 - Q_1)$$

4) Previous identified outliers were considered as extreme meteorological events for olive flowering. However, extreme meteorological events are considered only when meteorological outliers are present and in the year identified in point 2 (when the difference between typical PI and the biometeorological index >1).

Once each meteorological variable was identified and its outlier value involved in the extreme event, an adjustment factor was applied to optimise the biometeorological index concerned. We attempted to minimise the root mean square error (RMSE) between the observed typified PI and the typical biometeorological index using the following rule-based system of "bonuses" and "penalties":

1) Values previously identified as outliers were taken as the extreme values for weather variables.
2) A logical approach was adopted to establish whether the value of a variable in any year was higher than a given maximum extreme value or lower than a given minimum extreme value.
3) Where the value of a variable was higher than the maximum, a "penalty" (in the case of a negative event) or a "bonus" (for a positive event) was applied to the relevant biometeorological index. The same procedure was applied where the value of the variable was lower than a given minimum.
4) The values of "bonuses" and "penalties" were the results of minimising the RMSE between the typified biometeorological indices and the typified PI. Adjustment rules were applied to biometeorological indices prior to typing.
5) The "bonus" was applied by adding a value to the relevant biometeorological index in those years in which the extreme weather event took place; conversely, the "penalty" was applied by subtracting a value from the biometeorological index.

Optimization procedure

"Bonuses" and "penalties" obtained to minimise the RMSE were applied to the indices for the following adjustment factors:

(1) Adjustments to the DHI:

$$DHI_0 = (RfS \leftrightarrow J)/(HSunS \leftrightarrow J)$$

$$DHI_1 = \begin{cases} DHI_0, if(DRfS \leftrightarrow J) < 25 \\ DHI_0 + 85, if(DRfS \leftrightarrow J) \geq 25 \end{cases}$$

For any year with over 25 days of rainfall between September and January (DRfS↔J), a bonus of 85 was applied to the DHI.

b) Adjustments to the TI:

$$TI_0 = (minTM)/(RanTM)$$

$$TI_1 = \begin{cases} TI_0, if(minTJ) > 0.1 \\ TI_0 - 0.21, if(minTJ) \leq 0.1 \end{cases}$$

For any year in which minimum January temperatures (minTJ) were lower than 0.1°C, a penalty of −0.21 was applied to the TI.

$$TI_2 = \begin{cases} TI_1, if(minTJ) < 7.2 \\ TI_1 - 0.35, if(minTJ) \geq 7.2 \end{cases}$$

For any year in which minimum January temperatures (minTJ) were higher than 7.2°C, a penalty of −0.35 was applied to the TI.

$$TI_3 = \begin{cases} TI_2, if(minTM) < 9.6 \\ TI_2 - 0.59, if(minTM) \geq 9.6 \end{cases}$$

For any year in which the mean minimum temperature in March (minMT) was lower than 10°C, penalty of −0.59 was applied to the TI.

Building of a cyclicity index

The cyclicity index (CI)—an autoregressive index genrated using a previous database—enabled us to make an indirect estimation taking into account the influence of cyclical climate behaviour, to be incorporated in the model.

The CI was constructed using pollen data from 1988 to the present, since prior to 1988 the pollen production pattern displayed clear 2-year cycles.

In building the CI, the term "crest" was used to denote the change from a rising to a falling trend in the PI time series, while "valley" referred to the change from a falling to a rising trend.

The first step was to determine the number of years elapsing since the last appreciable crest (AC) and the number of years elapsing since the last appreciable valley (AV). The number of years since the last valley was taken as 0, provided that no rising trend was appreciated in the time series. CI_1 was taken as the sum AC and AV.

$$CI_1 = AC + AV$$

CI_2 included CI_1 and the pollen indices for previous years. The time series for pollen indices shows that a considerable increase in pollen counts with respect to the preceding year marked a change of trend.

$$CI_2 = \begin{cases} CI_1, if\ (PI_{n-1}) < \left(\frac{PI_{n-2}}{1.25}\right) + (PI_{n-2}) \\ \frac{CI_1}{2}, if\ (PI_{n-1}) \geq \left(\frac{PI_{n-2}}{1.25}\right) + (PI_{n-2}) \end{cases}$$

Where pollen counts for the previous year were over 180 % higher than those recorded the year before that, a change of trend was marked, and CI_1 was reduced by half.

$$CI_3 = (CI_2) - (CI_{2,n-1})$$

CI_3 was thus equivalent to CI_2 minus CI_2 for the preceding year, thus providing information on the estimated trend at any given moment in the time series.

$$CI_4 = \begin{cases} 1, if & CI_3 > 0 \\ 0, if & CI_3 = 0 \\ -1, if & CI_3 > 0 \end{cases}$$

To obtain CI_4, estimated rising trends were taken as 1 and falling trends as −1.

$$CI = \begin{cases} \left(\frac{PI_{n-1}}{4.8}\right) + PI_{n-1}, if\ CI_4 = 1 \\ PI_{n-1}, if\ CI_4 = 0 \\ \left(\frac{PI_{n-1}}{1.8}\right) - PI_{n-1}, if\ CI_4 = -1 \end{cases}$$

The final stage consisted of forecasting pollen counts on the basis of the PI for the previous year and the expected trend, i.e. the mean percentage year-on-year difference over the time series.

Building of Forecasting Models

Once the five indices were defined, two multivariate regression models were constructed to forecast the PI as a dependent variable. The meteorological-based model took simple weather variables as independent variables, whilst the index-based model included the four biometeorological indices and the CI as independent variables.

The performance of the models (confidence intervals, significance levels and standard errors) was compared, and they were also validated using bootstrap method. Bootstrap is a resampling technique used to create confidence intervals for coefficients concerned by performing 1,000 different regressions. The bootstrap can be used to derive accurate standard errors, confidence intervals, and hypothesis tests for most statistics. The sampling distribution of a statistic is constructed empirically by resampling from the sample. The resampling procedure is designed to parallel the process by which sample observations were drawn from the population. The key bootstrap analogy is the following: the population is to the sample as the sample is to the bootstrap samples. Bootstrap regression method has been programmed by R programming language. All variables were typified before bootstrapping to compare the sturdiness of the models to each other. Finally, the performance of the models (bootstrap confidence intervals, significance levels of models and standard errors of models) was compared.

Results

Preliminary correlation analysis

The results of a preliminary correlation analysis between the different meteorological-related variables and the PI indicated that the meteorological conditions in March, both temperatures and rainfall, were highly correlated with olive PI for the study period (Table 1). Regarding summer conditions, minimum temperature and sunshine hours showed a significant positive influence on PI. Finally, autumn–winter hydric conditions also showed a high correlation in Table 1. In general wet periods (i.e. 2003 and 2010) were associated with higher PI, and dry periods (i.e. 1993 and 2005) with lower PI.

Final indices

The final indices are presented in Fig. 1, which shows the good relationship between PI and the four optimized biometeorological indices.

The CI result is also presented in Fig. 1. The strong correlation between PI and CI is clear, as is the close fit of the two indices.

Table 2 shown the correlation matrix between all indices and the PI.

Models

Meteorological-based model (MM):

$$PI_{MM} = -10916.7 + 3152.31 \times (minTM) + 96.57 \times (RfF)$$

$R^2 = 0.47;\ p = 0.000$

minTM (minimum March temperatures); RfF (February rainfall)

The variables exerting the strongest effect on the PI were minimum March temperatures and February rainfall. MM standard error was 6818. Minimum temperatures for March and February rainfall are the variables that most affected the model; others that do not contribute much have been eliminated from the model.

Index-based model (IM):

$$PI_{IM} = 1556.78 + 0.696 \times (CI) + 18666.97 \times (TI) + 11.41 \times (PFHI) + 20.58 \times (SI) + 3.43 \times (DHI)$$

$R^2 = 0.81;\ p = 0.000$

IM standard error was 4,288.

The robustness of the two models was tested by means of a bootstrap validation. Confidence intervals at 95 % for the different coefficients obtained by bootstrap resampling can be seen in Table 3.

The medium confidence range of IM coefficients was 0.464 while the medium confidence range of MM coefficients of 0.609, which indicates a highest robustness for the IM.

Table 1 Pearson correlation coefficients between pollen index (PI) and meteorological parameters during the years 1982–2010. *minTM* Average value of daily minimum temperatures in March; *RanTM* average value of temperature range in March,; *RfF-M* sum of total rainfall in February and March (mm),; *minTJn–S* average value of daily minimum temperatures in June, July, August and September; HSunJn–A average value of daily sunshine in June, July and August (hours); *HSunS–J* Average value of daily sunshine hours in September, October, November, December and January; *RfS–J* sum of total rainfall in September, October, November, December and January (mm)

	minTM (°C)	RanTM (°C)	RfF↔M (mm)	minTJn↔S (°C)	HSunJn↔A (hours)	HSunS↔J (hours)	RfS↔J (mm)
PI	0.483**	-0.316*	0.504**	0.453**	0.508**	-0.363*	0.263*

*Correlation coefficient is significant at the 0.05 level; ** Correlation coefficient is significant at the 0.01 level

The higher determination coefficient and the lower standard error detected for the IM indicated a stronger effectively of this model. Moreover, the validation test indicated its higher sturdiness. Figure 2 shows the differences, in percentage, between observed and predicted PI using the two models. In 1995, a very high error (%) was detected since this measure is highly dependent on the total amount of pollen and the total concentrations in this year were very low.

Discussion

Knowledge of the main factors influencing the marked year-on-year changes observed in olive flowering intensity is of considerable scientific, agricultural, environmental and public-health interest. Earlier research in this field found that rainfall and, especially, temperature over the months prior to flowering was one of the most influential meteorological-related factors (Galán et al. 2001b; Ribeiro et al. 2006). It has also been shown that temperature is the most influential factor controlling the most important characteristics of olive reproductive phenology, such as the onset date or the peak date (Galán et al. 2001a; Orlandi et al. 2010b). As it has been indicated by several authors (i.e. Fornaciari et al. 1997), meteorological variables recorded during the pollen season itself also had a considerable influence on PI. However, they have no real predictive value, and should therefore not be included in forecasting models.

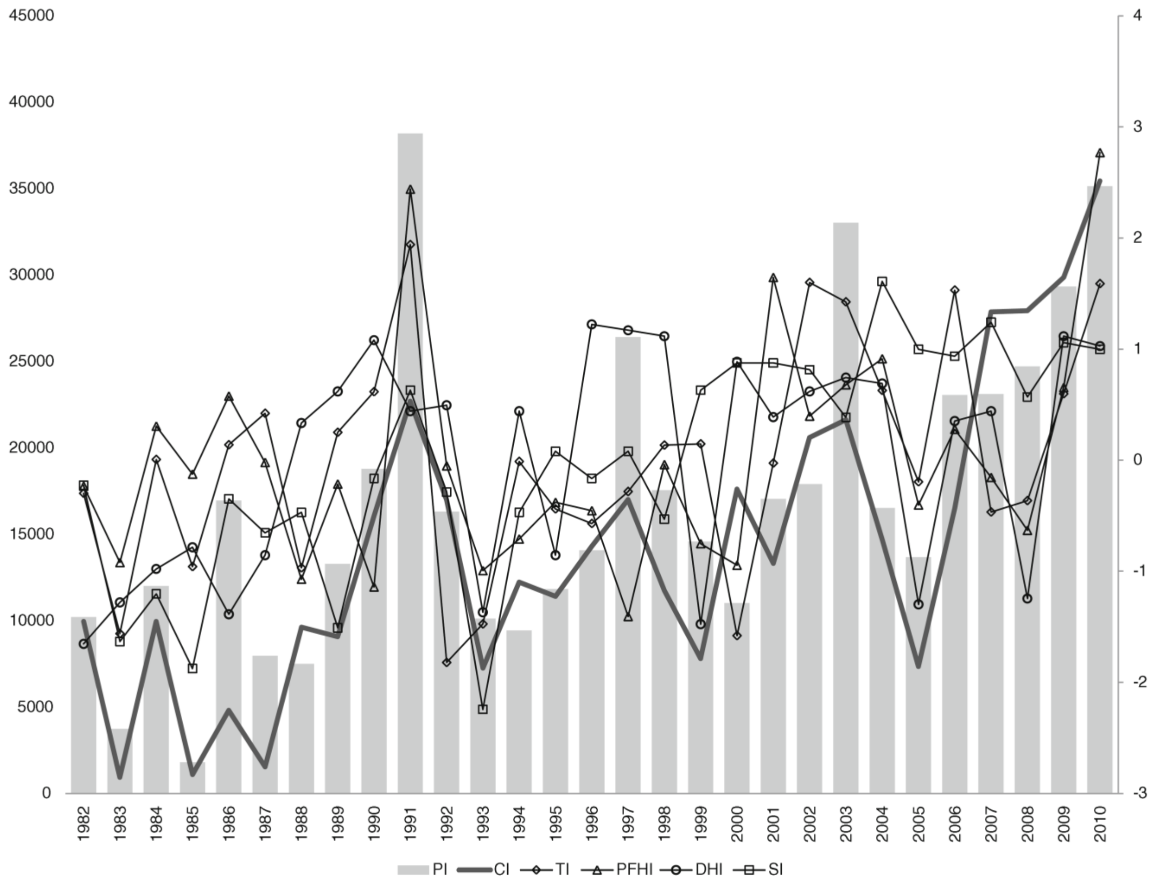

Fig. 1 Pollen index (PI), typical biometeorological indices and cyclicity index (CI)

Table 2 Pearson correlation coefficients matrix applied to PI, biometeorological indices and cyclicity index (CI) during the studied years 1982–2010. *TI* Thermal index, *PFHI* pre-flowering hydric index, *DHI* dormancy hydric index, *SI* Summer index

	TI	PFHI	DHI	SI	CI	PI
TI	1					
PFHI	0.65*	1				
DHI	0.32	0.22	1			
SI	0.47**	0.40*	0.37	1		
CI	0.43*	0.45*	0.56**	0.66**	1	
PI	0.63**	0.50**	0.47**	0.60	0.83**	1

*Correlation coefficient is significant at the 0.05 level, ** Correlation coefficient is significant at the 0.01 level

Although PI is one of the most important phenological and aerobiological characteristics, only a few studies have sought to propose forecasting models to predict PI in different species, for example *Olea* (Galán et al. 2001b; Ribeiro et al. 2006), *Betula* (Dahl and Strandhede 1996), *Quercus* (Corden and Millington 1999) and *Poaceae* (Emberlin et al. 1999; García-Mozo et al. 2010b). This small number of studies could be explained by the difficulty of proposing accurate PI predictions due to the complexity of factors influencing it. Although in general PI-forecasting studies have included simple meteorological variables in the proposed models, some studies have already suggested that bioclimatic indices could provide more information regarding year-on-year changes in the PI, thus affording a sound basis for the creation of forecasting models (e.g. Valencia-Barrera et al. 2002).

The present study sought to propose a biometeorological forecasting model based on biometeorological indices that would reflect the meteorological conditions affecting the physiological status of olive trees before flowering starts. The four proposed biometeorological indices were optimised, taking into account extreme meteorological events characteristics of the Mediterranean climate. An autoregressive CI was also developed. The model is adapted exclusively to the study area. The introduction of all the "bonuses and penalties", the consequent RMSE minimisation process, and the introduction of the CI all combine to make the model very effective. However, the model can be exported to other areas only with previous accurate knowledge of both meteorological conditions and historical yields.

In constructing the biometeorological indices in this work, a distinction was made between heat-related and water-related variables, since their effects may differ considerably from each other depending on season and phenological stage. This distinction is essential in order to ensure the value of these indices for future research.

March temperatures and rainfall exerted considerable influence on the PI. This result agrees with earlier studies in Córdoba province (Galán et al. 2001b). However, excessively high temperatures—such as those recorded in 2001—can lead to heat stress; accordingly, it was necessary to apply a penalty to the TI. Elsewhere, mean and minimum temperatures from January to March—as well as autumn rainfall—are reported have a major effect on olive flowering (Ribeiro et al. 2006). The strong influence of March temperatures and rainfall on flowering phenology has also been noted for other tree species in temperate climates (García-Mozo et al. 2001).

Regarding extreme abnormal temperature events—although the olive requires a winter chilling period in order to break dormancy, excessively low temperatures will have a negative impact (Bartolozzi and Fontanazza 1999), or at least will not favour the breaking of dormancy (Orlandi et al. 2004). Therefore, for any year in which minimum January temperatures are low, a penalty was applied to the TI.

In Mediterranean climates, spring-flowering tree species require a certain number of days with low temperatures to ensure the heat stress required to break dormancy. The olive needs a minimum number of chilling hours below a threshold temperature of 7.2°C prior to budburst (Aron 1983). For that reason, for any year in which minimum January temperatures were higher than 7.2°C, TI was optimized.

The importance of summer conditions for PI can be explained by the fact that the rate of flowering-bud differentiation after dormancy is influenced heavily by summer weather conditions. Summer temperatures are known to have a major impact on flowering in summer-budding tree species, abnormally high summer temperatures often give rise to abundant pollen in the following spring, (Mandrioli

Table 3 Bootstrap validation summary. Confidence intervals at 95% for the different coefficients obtained by bootstrap re-sampling. *UP* Upper limit, *LL* lower limit, *R* Range

Index-based model				Meteorological-based model			
Coefficient	LL (95%)	UL (95%)	R (95%)	Coefficient	LL (95%)	UL (95%)	R (95%)
Constant	-0.181	0.167	0.348	Constant	-0.279	0.292	0.571
CI	0.437	0.945	0.508	MinTM	0.174	0.752	0.578
TI	0.107	0.610	0.503	RfF	0.078	0.756	0.678
PFHI	-0.225	0.429	0.654				
SI	-0.241	0.171	0.412				
DHI	-0.195	0.166	0.361				

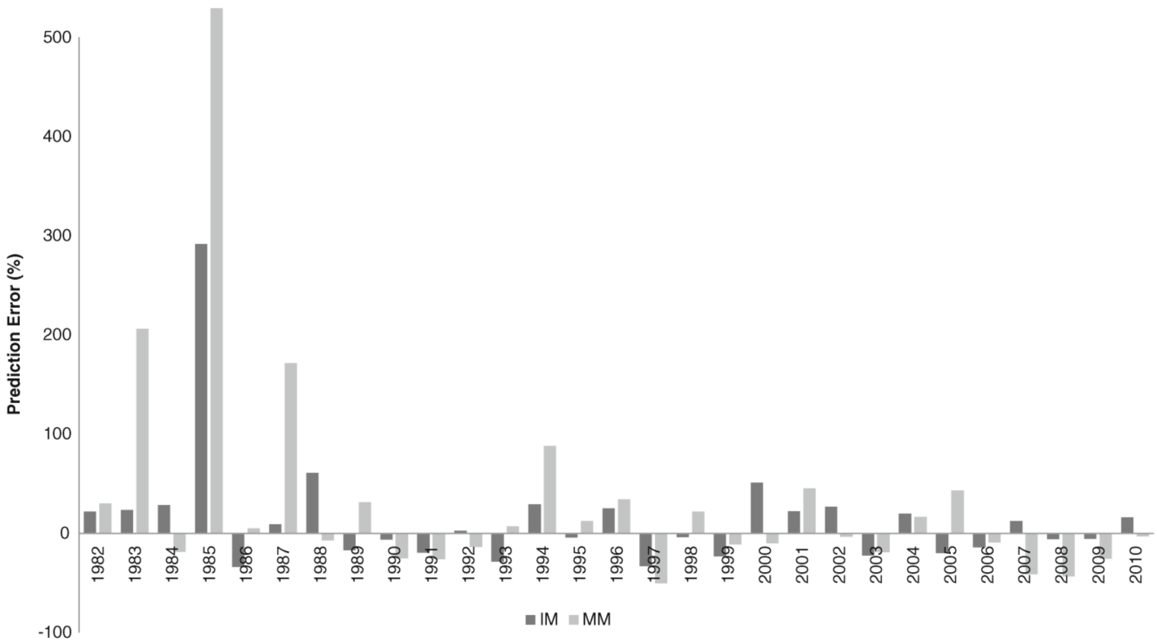

Fig. 2 Differences (in percentage) between observed and predicted PI values. IM Index-based model, MM meteorological-based model

1987). In the case of olive, the rate of flowering-bud differentiation is affected strongly by the prevailing weather conditions over the previous summer (Rallo and Cuevas 2004). A significant correlation was recorded here between the SI and the PI, although other authors report that summer weather conditions tend to correlate more strongly with fruit development than with the PI (Galán et al. 2008; Ribeiro et al. 2008).

It is known that precipitation-induced increase of water availability to the plant and hours of sun are related inversely—a fact corroborated by our results. Water availability is dependent directly on the amount of rainfall; nevertheless, temporal distribution of precipitation is also very important. Moderate rainfall ensures greater water availability for the plant; in contrast, we noted that heavy rainfall concentrations lead to an excessive DHI, and this index is therefore less representative than if rainfall had been lighter and regular. Other authors have also noted that number of days' rainfall is a key factor in plant development (Ribeiro et al. 2006). In this sense it was also observed that in years recording the highest level of 25 days rainfall between September and January, indices were optimised (which prompted us to give a bonus to the DHI).

Since species tend to be well-adapted to their native areas, the relationship between plant development and habitual local weather-related variables appears to be linear. But in fact weather variables do not act on plant development in a linear manner: each species has its own optimum range of values for each variable, outside of which development is less successful. If a variable takes on values that are very high or very low, and cause equally negatives effects on plant development, is becomes impossible to appreciate global behaviour using a linear relationship. The use of linear models in bioclimatological research thus provides little useful information on non-linear weather phenomena; optimisation seeks to overcome that deficiency. Therefore we considered that it would be important to optimise indices using certain adjustment rules, simply because there is no linear correlation between the variables involved in extreme weather events and the PI. In the revised literature, there are no studies taking into account the effect of extreme weather conditions on PI.

Although time series trends are not usually included in the models, we notice a rising trend on olive pollen concentration at Córdoba province during recent years. This could be due to the fact that, during the study period, the area of olive planting in Córdoba increased by 20.7 % (71,190 ha) and irrigation increased by 92.7 % (16,576 ha) (Agriculture Statistical Yearbook, 1982; 2010). Moreover, the study period also saw a change in weather conditions that may have influenced the trend towards a higher PI.

In order to take this trend into account, an autoregressive CI was also created. A new feature of the present study is the use of this autoregressive method—the CI—for PI forecasting. Other authors have used autoregressive models to forecast daily pollen counts, but not hitherto for long-term PI predictions, perhaps because of the long time-series required to construct models of this kind (Arca et al. 2002; Belmonte and Canela 2002; Rodríguez-Rajo et al. 2006). When grouping years with a view to determining cyclicity, it should be borne in mind that olive-tree also have an alternate bearing cycle that is influenced by a number of factors, including individual tree age, cultivar and climate. Alternate bearing is an evolutionary strategy through which the tree produces a large amount of seeds in some years, thus enhancing its biological efficiency. Resources are stored over one year and invested in the following year. However, crop practices adopted since the mid-1980s have reduced the effect of the alternate bearing cycle, and have favoured a general increasing trend (Domínguez et al. 1993b). Although the PI time-series used to generate the CI comprised only 29 data points, this were sufficient for the present purpose, i.e. forecasting the trend of the time series. This CI also provides an indication—albeit indirect—of climate conditions in previous years in that it is based on historical pollen indices. Since the plant contains varying levels of reserve substances that are stored from one year to the next, spring flowering intensity is influenced strongly by prior productive status, which is in turn dependent on previous weather conditions (Ramos 2000). The CI also reflects changes in the alternate bearing cycle, although the effect of alternate bearing has been diminished through the adoption of new cropping techniques. Moreover, CI provides information on cyclical variations characteristic of the Mediterranean climate, consisting chiefly in the alternation of wet and dry periods.

The combination of all the methodology proposed in this paper provides a useful tool for prediction in bioclimatology. The use of an autoregressive index and a method for optimizing biometeorological indices in the face of extreme meteorological events have provided very appropriate tools with which to predict the IP. Future studies could use the proposed method to make more accurate predictions. The CI has an obvious advantage over the index-based model, therefore this paper should not be considered as a comparison between the efficacy of using biometeorological indices against simple meteorological variables but rather as a comparison between the effectiveness of a traditional model versus employment of the indices developed here. Finally, the comparison and validation of the models proved that the index-based model is an accurate model that performs better for olive pollen forecasting purposes than traditional meteorological-based model.

Conclusions

The four biometeorological indices provide the basis for future research into the weather-based characterisation of phenological years, for olive and potentially for other species as well.

The proposed method was developed taking into account the effects of extreme events, providing a sound basis for the improvement of phenological forecasting models in the Mediterranean area.

The CI, with its novel use of autoregressive techniques for predicting the PI, considerably improved forecasts. The autoregressive technique has not been employed previously in aerobiological research, and was in fact purpose-built for this study.

The index-based model provided more accurate forecasts than the traditional meteorological-based model, indicating that the use of optimised biometeorological indices together with a CI enhances the performance of prediction models.

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5. CAPÍTULO II

Year clustering analysis for modeling olive flowering phenology

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ORIGINAL PAPER

Year clustering analysis for modelling olive flowering phenology

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Abstract It is now widely accepted that weather conditions occurring several months prior to the onset of flowering have a major influence on various aspects of olive reproductive phenology, including flowering intensity. Given the variable characteristics of the Mediterranean climate, we analyse its influence on the registered variations in olive flowering intensity in southern Spain, and relate them to previous climatic parameters using a year-clustering approach, as a first step towards an olive flowering phenology model adapted to different year categories. Phenological data from Cordoba province (Southern Spain) for a 30-year period (1982–2011) were analysed. Meteorological and phenological data were first subjected to both hierarchical and "K-means" clustering analysis, which yielded four year-categories. For this classification purpose, three different models were tested: (1) discriminant analysis; (2) decision-tree analysis; and (3) neural network analysis. Comparison of the results showed that the neural-networks model was the most effective, classifying four different year categories with clearly distinct weather features. Flowering-intensity models were constructed for each year category using the partial least squares regression method. These category-specific models proved to be more effective than general models. They are better suited to the variability of the Mediterranean climate, due to the different response of plants to the same environmental stimuli depending on the previous weather conditions in any given year. The present detailed analysis of the influence of weather patterns of different years on olive phenology will help us to understand the short-term effects of climate change on olive crop in the Mediterranean area that is highly affected by it.

Keywords Olive · Phenology · Aerobiology · Forecasting model · Clustering · Climate change

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Introduction

This study was focussed on the southern Spanish region of Andalusia, which has by far the world's largest area given over to olive plantations (1,511,687 ha) and an annual olive output generally exceeding 5,000,000 t. Within this region, Cordoba province has the second-largest olive-growing area, with 343,812 ha producing an average of 1,000,000 t olive crop (Andalusia Statistical Yearbook 2010).

The olive is a temperate, spring-flowering tree. The bioclimatic requirements for flowering vary as a function of the tree's phenological status (Galán et al. 2001b; Aguilera and Ruiz 2009). Reproductive structures grow from undifferentiated buds within a few months of dormancy, this phase is termed differentiation. But differentiation must occur after induction: certain temperature requirements need to be met in order for bud break to take place; these may vary depending on the olive variety and on the degree of climate adaptation (Galán et al. 2005; García-Mozo et al. 2009). Although it is generally accepted that an initial induction occurs during summer months, a stress period during winter (induced by low temperatures) is also required to break bud dormancy (Andreini et al. 2008; Fernandez-Escobar et al. 1992; Orlandi et al. 2004; Rallo and Martin 1991). Flowering starts once a certain amount of heat has been accumulated but cold spells during endodormancy have been found to favour increased inflorescence formation, while the lack of cold leads to further development of branches

(Bonofiglio et al. 2009; Orlandi et al. 2005; IOOC 1996). In general, the Mediterranean climate is characterised by hot, dry summers and mild, rainy winters. However, it is also characterised by a marked year-on-year variations in weather patterns, due mostly to the alternation of wet and dry year periods.

Because olive trees are anemophilous, flowering intensity was measured by means of airborne pollen detection. The amount of pollen detected by air samplers is considered to be direct proportional to the flowering intensity in anemophilous species that are within the effective area of the pollen sampler (Frenguelli 1998; Subba-Reddi and Reddi 1985). However, this assumption should be treated with some caution since, before being caught by the air sampler, the pollen has had to surmount the release process and transport to the pollen sampler, processes highly dependent on environmental conditions (García-Mozo 2011; Frenguelli 1998; Subba-Reddi and Reddi 1985). Weather conditions influence the flowering season, not only by regulating flower and pollen production but also, in the case of wind-pollinated plants, by regulating the timing of pollen season and therefore shaping pollen-curve dynamics. Olive is also characterised by exhibiting a high tendency toward variable flowering intensity that is highly related to the alternation in fruit production (Galán et al. 2004, 2008; Lavee 2006). Earlier research in the study area showed that olive flowering intensity was influenced mainly by temperature and rainfall over the preceding months (Galán et al. 2001a). Olive flowering may also vary, even within the study area, as a function of plant physiological status, which is itself governed by a number of factors including genetic background, internal reserves and local weather conditions (García-Mozo et al. 2009). The present study sought to test the hypothesis that the differentiation of year categories as a function of phenological and meteorological features, prior to the construction of phenological flowering-intensity models, would help to render these models more accurate. The different categories would serve as indicators of physiological status, as well as providing value information on reproductive phenology. Clustering analysis enabled different weather-related variables to be included in each category. It was assumed that year-clustering would provide a better fit for phenological models. For this purpose, the following three-step method was developed: (1) a clustering step using a K-means method, (2) a classification step using an artificial neural network (ANN) method, and (3) a forecasting step using the partial least squares regression (PLSR) method. Since olive is wind-pollinated, it was possible to precisely measure flowering intensity as a pollen index (PI) that records the sum of daily airborne pollen data throughout the entire pollination season (Galán et al. 2007).

In an earlier paper, we constructed some indices covering the main variables affecting olive flowering intensity in Cordoba: the thermal index (TI), the pre-flowering hydric index (PFHI), and the cyclicity index (CI) (Oteros et al. 2012). These were constructed using weather-related and phenological data, and various adjustment criteria were tested to ensure that models reflected the extreme weather events characteristic of the Mediterranean climate. These indices were used for clustering purposes in the present study. A number of authors have made use of clustering techniques in phenological and aerobiological research, e.g. for pollen back-trajectory analysis in the atmosphere (Hernández-Ceballos et al. 2011), and for daily airborne pollen forecasting (Makra et al. 2006; Sánchez-Mesa et al. 2002). The year-clustering approach has also been used in comparing classification methods and for general long-term forecasting, although not hitherto for specific, long-range forecasting purposes (Sánchez-Mesa et al. 2005). In the present study, each proposed year category would represent a distinct physiological situation, resulting from exposure of olive trees to different environmental conditions. With a view to selecting the model providing the best classification of future years in terms of weather-related features, several classification methods were tested. Fitted models to predict olive flowering intensity constructed for each year category were compared to a general model including all the years of the studied period.

This study should improve our knowledge of olive reproductive behaviour in a Mediterranean climate, i.e. in a region specially affected by climate change (IPCC 2007). Plant phenology is seen as one of the most important bio-indicators of climate change, since trends can provide considerable temporal and spatial information regarding ongoing changes (Galán et al. 2005). Analysis of the influence of weather patterns on olive phenology will help us to understand the short-term effects of climate change on olive crops. Also, advance information regarding olive-pollen intensity could be of particular value in a number of fields. Olive pollen is highly allergenic in the Mediterranean area (Dominguez et al. 1993; D'Amato et al. 2007; Barber et al. 2008), and patients with atopic or allergic asthma could be forewarned regarding likely pollen peaks. Pollen counts also provide a valuable bioindicator of flowering intensity and therefore of the volume of the forthcoming harvest, and are thus of value to farmers (Galán et al. 2004, 2008; García-Mozo et al. 2008; Ribeiro et al. 2008).

The first aim of this study was to develop a method for forecasting olive pollen season intensity by clustering years on the basis of phenological and meteorological data. A second objective was to compare the accuracy between different classification methods trying to improve the methodology.

Materials and methods

Study area and phenological data

The city of Cordoba is situated in south-west Iberian Peninsula (37°50′N, 4°45′W), 123 ma.s.l. The area has a Mediterranean climate with some continental features. The annual mean temperature is 17.8 °C and the annual average rainfall is 621 mm. Phenological data for the last 30 years (1982–2011) regarding flowering intensity were measured by analysing airborne pollen using a Hirst-type volumetric spore trap (Hirst 1952) placed on the roof of the Educational Sciences Faculty, at 15 m above ground level, which offers olive pollen data from a radius of 100 km around, well representing Cordoba province olive flowering phenology (Hernández-Ceballos et al. 2011). Pollen counts were obtained using a standard protocol published by the Spanish Aerobiology Network (REA) (Galán et al. 2007). Weather data for the last 30 years (1982–2011), provided by the Spanish State Meteorology Agency (AEMET), were taken at Cordoba Airport, located around 5 km south of the pollen-sampling site.

Year clustering

The first step was to assign the study years into clusters, on the basis of phenological and weather data. Phenological features of the intensity of flowering season were based on the variations of PI and average daily pollen counts during the pre-peak period of the pollen season (ADPP). Three biometeorological indices, proposed in an earlier study (Oteros et al. 2012), aimed at identifying the factors most influencing pollen season characteristics: TI (1), which takes into account the heat conditions in January and March by adjustment criteria; PFHI (2), which takes into account rainfall in February and March (both indices covering the heat and water conditions most affecting olive flowering intensity in Cordoba); and finally CI (3, 4), an autoregressive index that seeks to emulate cyclic changes in the pollen-index time-series. The development of the CI is summarized in two steps (3, 4), where CI_2 is the final CI.

$$TI = (T\min M)/(RanTM) \tag{1}$$

(TminM) is minimum March temperatures and (RanTM) is March temperature range.

$$PFHI = (RfF) + (RfM) \tag{2}$$

(RfF) is the sum of total rainfall in February and (RfM) is the total rainfall in March.

$$CI_1 = \begin{cases} AC + AV,\ if(PI_{n-1}) < \left(\frac{PI_{n-2}}{1.25}\right) + (PI_{n-2}) \\ \frac{AC+AV}{2},\ if(PI_{n-1}) \geq \left(\frac{PI_{n-2}}{1.25}\right) + (PI_{n-2}) \end{cases} \tag{3}$$

(AC) is the number of years elapsing since the last appreciable crest and (AV) is the number of years elapsing since the last appreciable valley. The term “crest” was used to denote the change from a rising to a falling trend in the PI time series, while “valley” referred to the change from a falling to a rising trend (Oteros et al. 2012).

$$CI_2 = \begin{cases} \left(\frac{PI_{n-1}}{4.8}\right) + PI_{n-1},\ if(CI_1) - (CI_{1_{n-1}}) > 0 \\ PI_{n-1},\ if(CI_1) - (CI_{1_{n-1}}) = 0 \\ \left(\frac{PI_{n-1}}{1.8}\right) - PI_{n-1},\ if(CI_1) - (CI_{1_{n-1}}) < 0 \end{cases} \tag{4}$$

Clustering analysis

First, the optimum number of natural groups of years (K) was determined by hierarchical clustering analysis; clusters were then generated by “K-means” conglomerate analysis. Both methods were developed using the software Unscrambler 9.7 (http://www.camo.com).

Hierarchical clustering analysis was performed using Ward’s method, in which information is quantified as the sum of squared distances of each element with respect to the centroid of the cluster to which it belongs. To do this, we first calculated the mean vector of all variables, the multivariate centroid for each cluster. Next, we calculated the squared Euclidean distances between each element and the centroid (mean vector) of all clusters. Finally, distances for all elements were combined.

The “K-means” conglomerate method was used for cluster generation: “k” groups of years were generated on the basis of similar meteorological and phenological characteristics. The five variables used were standardised before performing cluster analysis, in order to remove any dependency on measurement units. Of the various types of cluster analysis available, this was deemed to the most appropriate, in that it provides a more flexible approach and does not assume any specific distribution of variables.

Characterisation of year categories

To characterise each category, the centroid of each cluster was analysed; weather and pollen-count patterns were examined for each of the years making up the cluster. The four year categories were designated C1–C4. A descriptive statistic analysis of several features of the years was performed using the software R 2.11.1. We analyzed the averages and standard deviations of meteorological parameters and aerobiological features of years.

Classification modelling

Models were constructed to classify other years on the basis of the weather conditions recorded prior to the pollen season, using the indices TI, PFHI and CI. Classification models were constructed using three different techniques: (1) linear discriminant analysis, (2) decision trees and (3) artificial neural networks.

The models were validated using the k-fold cross-validation test. K-fold cross-validation is similar to simple cross-validation but the original sample is partitioned randomly into k subsamples. Of the k subsamples, a single subsample is retained as the validation data for testing the model, and the remaining k−1 subsamples are used as training data. The cross-validation process is then repeated k times (the folds), with each of the k subsamples used exactly once as the validation data. The optimal k used was 4 and that is the most appropriate k number considering that we work with four categories.

All classification models were built using the software Weka 3.7.4 (http://weka.softpedia.com/).

Linear discriminant analysis

The goal of linear discriminant analysis (LDA) is to establish a linear discriminant function, based on the original variables, which will discriminate between different classes. LDA seeks a linear combination or function, D, of the independent variables that maximizes the between-class variance relative to the within class variance. The latent variable thus obtained is called a canonical variate. For k classes, k−1 canonical functions can be calculated. Each time, LDA will select a direction leading to maximum discrimination between the given categories (Fisher 1936).

Decision trees

Decision tree (DT) building is a machine-learning method adapted for classification and prediction. DT-based methods express their results as a graphical presentation of decision rules. DT consists of a root, a number of internal nodes representing attributes and a sequence of branches representing attribute values. The tree ends in leaves reflect the appropriate target attribute and indicating a class. The descending order of the attribute is calculated on the basis of the gain ratio. The attribute with the highest information gain is selected for generating the root of the tree (Kirchner et al. 2004). The algorithm selected in our work as the most accurate was the termed LMT-I-1-M 15-W 0.0 using Weka 3.7.4.

Artificial neural network

In this subsection we consider standard sigmoidal ANN, or multilayer perceptron (MLP), as the base classification model. ANN can overcome the longer training time and the difficulty in determining hidden layer units of a backpropagation network to a large extent. An ANN is a three-layer feed-forward neural network. For determining the best ANN model, we apply an backpropagation algorithm to find the basis functions or nodes of the hidden layer: $B(x,W)=\{B_1(x,w_1), B_2(x,w_2), \ldots, B_m(x,w_m)\}$, corresponding to the nonlinear part of the discriminant functions, $f_l(x,\theta_l)$. We have to determine the number of basis functions "m" and the weight matrix "w". To apply evolutionary neural network techniques, we consider an ANN with softmax outputs defined in Eq. (5) and the standard structure: an input layer with a node for every input variable; a hidden layer with several sigmoidal nodes; and an output layer with J-1 nodes, where J is the number of classes.

If the output layer of the $ANN\left(\widehat{\theta}\right)$ classifier is interpreted from the point of view of probability, which considers the softmax activation function:

$$g_1(x,\theta_l) = \frac{\exp f_l(x,\theta_l)}{\sum_{l=1}^{Q} \exp f_l(x,\theta_l)} for\, l = 1,\ldots,Q \quad (5)$$

where $g_l(x,\theta_l)$ is the probability a pattern x has of belonging to class l, $\theta_1 = (\beta_1, w_1, \ldots, w_M)$, where $\beta_1 = \left(\beta_1^1, \ldots, \beta_M^1\right)$ is the l-th vector of weights of a node in the hidden layer to the l output node, M is the number of hidden nodes, $w_j = \left(w_0^j, \ldots, w_k^j\right)$ for j=1......M, is the vector of weights of the input layer to the hidden node j, and $f_l(x,\theta_1)$ is the output of the l-th output node for pattern x given by:

$$f_l(x,\theta_l) = \beta_0^l + \sum_{j=1}^{M} \beta_j^l \beta_j\left(x, w_j\right) for\, l = 1,\ldots Q-1 \quad (6)$$

$$f_Q\left(x,\theta_Q\right) = 0 \quad (7)$$

where $B_j\left(x,w_j\right) = \sigma_j\left(w_0^j + \sum_{i=1}^{k} w_i^j x_i\right)$ is the sigmoidal activation function of the nodes in the hidden layer.

The classification rule coincides with the optimal Bayes rule. In this way, by g classifiers, the classification rule assigns an individual to the class with the maximum probability, given vector measurement x: $C(x) = \widehat{l}$, where $\widehat{l} = \arg\max_l g_l\left(x,\widehat{\theta}_l\right)$, for l=1,…,Q. Detailed descriptions of various forms of neural networks are provided elsewhere (Bishop 1995; Haykin 1994; Gutiérrez et al. 2009; Martinez-Estudillo et al. 2006).

Results of classifications models (the confusion matrices and the percentages of correctly classified years) were compared in order to select the most effective method.

Modelling

To analyse the performance of clustering analysis, different models were tested for each year category. The performance of these models (significance level, standard error and RMSE) was compared with that of other forecasting models not involving conglomerate analysis. RMSE was obtained by the following expression (8), where Y = observed data and F = expected data:

$$RMSE = \sqrt{\frac{\sum_{t=1}^{N} (Y_t - F_t)^2}{N}} \quad (8)$$

Forecasting models were constructed using the PLSR technique, taking PI, i.e. the annual sum of daily pollen counts, as dependent variable. Fitted predictive models were thus generated for each of the four categories (designated C1M to C4M), and a general model (GM) was constructed using all years. The models were built using the software Unscrambler 9.7.

Modelling was based on a linear transformation of the original descriptors to a small number of orthogonal factors (latent variables), attempting to maximize the covariance between the descriptors and the dependent variable; this procedure provides the optimal linear model in terms of forecasting. Here, each latent variable represented a key factor for olive flowering intensity.

Results

Clustering

To obtain the number of classes to be considered in the “K-means” algorithm, we applied hierarchical clustering, yielding the optimal number of groups that should be considered for “k-means” clustering equal to 4.

Once the optimal number of classes was defined, we applied a “4-means” clustering to group the study years into four clusters as a function of pollen-counts and weather-related characteristics. Categories were defined on the basis of meteorological characteristics and pollen parameters considered indicative of olive physiological and phenological status. PI is related directly to both types of parameters.

Centroid characteristics for each cluster are shown in Table 1; years belonging to a given category shared similar characteristics. The variables were bioclimatic indices, duly standardised in order to remove any dependency on measurement units. Figure 1 shows the relationship between years clustered into different categories and PI. In Fig. 1b we can see the structure of the dendrogram resulting from hierarchical cluster analysis.

Table 1 Centroids of each cluster. *CI* Cyclicity index, *PFHI* pre-flowering hydric index, *TI* thermal index, *PI* pollen index, *ADPP* average daily pollen counts during the pre-peak period of the pollen season

	Conglomerate			
	1	2	3	4
CI	−0.68	−0.36	0.57	1.52
PFHI	−0.53	0.47	−0.46	1.36
TI	−0.59	−0.07	0.27	1.29
PI	−0.83	−0.15	0.49	1.75
ADPP	−0.74	0.11	0.26	1.48

Category characterisation

Results for the descriptive analysis of years assigned to the various categories are shown in Table 2. Each category was defined in terms of the main features of the pollen season: the PI, pollen peak (PPk), pollen-season start-date (StD) and number of days with more than 100 grains (>100). Two major bioindices influencing the pollen season were included: PFHI and TI.

Weather-related variables for the months from January to May refer to the current pollen season, while variables for summer (June–September) and autumn (September–December) months refer to the previous pollen season, since these have a greater effect on flowering characteristics.

Category 1: Dry years

The years assigned to cluster 1 displayed lower PIs (55 % of the average), low daily pollen counts and low pollen peaks (Table 2). In terms of weather conditions, they were characterised by mild summers: maximum, mean and minimum temperatures from June to September were below the average for Cordoba. Autumns were dry and had a wider-than-average temperature range (12.5 ° C vs. average 11.9 °C). Rainfall between September and January in Category 1 (307 mm) was also lower than average for Cordoba (370 mm). The period from January to April was cold and dry, minimum temperatures being lower than average: January 0.8 °C (average 3.8 °C), March 0.7 °C (average 7.2 °C). May temperatures were 0.6 °C below average (27.1 °C). Rainfall from February to April was 25 % below average.

Weather data for 1982 lay at the shortest distance from the centroid of the cluster, signifying that they were the most representative for Category 1.

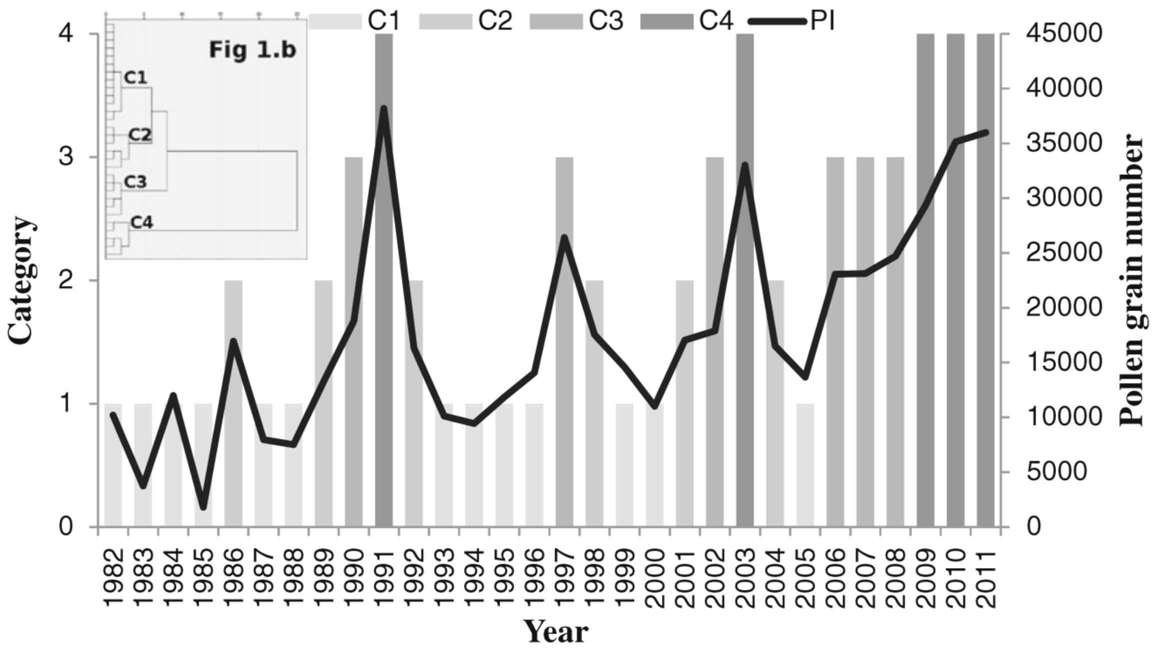

Fig. 1 Relationship between years clustered into different categories and pollen index (PI). Category 1 (C1), Category 2 (C2), Category 3 (C3), Category 4 (C4). *Inset* shows the dendrogram resulting from hierarchical clustering

Category 2: Cold years

Category 2 displayed average PIs and daily pollen counts. Like C1, however, the number of days with over 100 pollen grains was low. Summer temperatures were below the average for Cordoba. Maximum temperatures from October to December were 1 °C below average, while average temperatures were recorded in January and February; April temperatures were 1.1 °C below average. Rainfall during this period was within the average range for the area.

Category 3: Warm years

PIs in Category 3 years were 25 % above average, while daily pollen counts were average; however, the number of days with pollen counts exceeding 100 pollen grains/m^3 of air was higher than average. In Category 3 years, flowering started 5 days earlier than average. Summer and autumn temperatures were average, while rainfall from September to January was 80 mm above average. Temperatures from January to April were higher than the average for Cordoba: maximum temperatures from January to March were around 1 °C above average. Rainfall during this period was within the average range for the area.

Category 4: Wet years

Category 4 was characterised by very high PIs (193 % of local average), and high daily pollen counts. The pollen peak was higher than in the other categories. The pollen season was longer and the number of days with pollen counts exceeding 100 pollen grains/m^3 of air was greater.

Table 2 Descriptive analysis of each category (C1–C4). Pollen season characteristics: *PI* Pollen index, *PPk* pollen peak, *StD* starting date, *>100* number of days with more than 100 grains. Some important biometeorological indices influencing pollen season were included: *PFHI* pre-flowering hydric index, *TI* thermal index

	C1		C2		C3		C4		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PI	9,841	3,832	16,277	1,526	22,332	3,338	34,343	3,356	17,710	9,500
PPk	1,512	1,146	2,292	1,099	2,702	854	3,901	2,040	2,304	1,485
StD	115	7	114	13	108	13	116	9	113	10
>100	25	11	26	7	32	5	31	7	27	9
PFHI	67	32	140	53	72	55	205	87	106	73
TI	0.37	0.11	0.45	0.14	0.54	0.16	0.72	0.10	0.48	0.18

Summers were characterised by extreme temperatures: maximum, mean temperatures and minimum temperatures were 0.6 °C above average.

Autumns and winters were mild and rainy: the temperature range from September to January was 0.9 °C below average. Rainfall was higher than average over this period. The temperature range from January to April was 0.8 °C below average. Rainfall from February to April was 80 mm higher than the average for Cordoba.

Classifications

Three different methods were used to classify years within a given class: (1) discriminant analysis; (2) decision trees; and (3) neural networks.

Three variables were used for to test each analysed method: TI, PHI and CI. The three models were validated by 4-fold cross-validation. A total of 73.3 % of cases were classified correctly by LDA, a total of 76.6 % of cases were correctly classified by the DT method and a total of 80 % of cases were correctly classified by the ANN method. The neural network model thus proved to be the most effective of the three models tested. Table 3 shows the confusions matrices resulting from the classification methods tested.

Forecasting modelling

Different PLSR models were constructed to predict PI. A model was generated for each year category (C1M, C2M, C3M and C4M), and one GM was constructed for all study years.

A summary of statistical parameters, including R^2 and RMSE for the training model and cross-validation for the PI model, is shown in Table 4. As the results indicate, differentiation into several categories could be a useful tool for forecasting modelling.

The relationship between observed PI and the PI predicted by each PLSR model is shown in Fig. 2, which shows the PI predicted by the GM and the PI predicted by each specific category model.

The major coefficients for each model are shown in Fig. 3. The influence exerted by the weather-related variables examined here varied among categories. In overall terms, coefficients show that the general model of conditions prompting high PI included hot summers, rainy autumns, cold winters and mild, rainy springs. Category 1 years, characterised by drought, cold and low flowering intensity, were dependent largely on March rainfall and did not require lower winter temperatures. Category C2 years—with lower-than-average temperatures and medium-to-low flowering intensity—also had no need for very low winter temperatures. In C3 years, characterised by warm temperatures and middle-high flowering intensity, the high minimum temperatures in March had a negative effect. Coefficients for C4 years, which had warm temperatures, heavier rainfall and high flowering intensity, were similar to those recorded for the GM.

Discussion

This study sought to identify the weather-related parameters most influencing olive flowering intensity, expressed as PI, in the Mediterranean climate. Although in anemophilous plants PI is a good indicator of flowering intensity, this statement should be taken with some caution since before the pollen can be caught by an air sampler, it has had to surmount the release process and be transported to the pollen sampler—processes highly dependent on environmental conditions (García-Mozo 2011; Frenguelli 1998; Subba-Reddi and Reddi 1985). Nevertheless PI offers the possibility of obtaining a quantitative value for a wide area of study that can be compared objectively year by year.

A knowledge of factors most affecting flowering variations is of particular value for agricultural and environmental studies, and also for allergy sufferers, in the Mediterranean area due to its variable climatic characteristics. A great deal of research has attempted to determine the factors affecting phenology and flowering intensity in plant species, placing special attention on the effect of climate change (e.g. Linkosalo et al. 2010;

Table 3 Confusion matrices. Results of several classification methods: *LDA* Linear discriminant analysis, *DT* decision trees, *ANN* artificial neural network

		LDA				DT				ANN				
		Predicted category				Predicted category				Predicted category				Total
		1	2	3	4	1	2	3	4	1	2	3	4	
Observed Category	1	10	0	2	1	12	1	0	0	10	2	0	0	13
	2	0	4	0	1	4	2	0	0	3	3	0	0	5
	3	1	0	3	2	1	0	5	0	0	0	6	0	6
	4	1	0	0	5	0	0	1	4	0	0	1	4	6

Table 4 Pollen index (PI) partial least squares regression (PLSR) model summary. *GM* General model, C_1M category 1 model, C_2M category 2 model, C_3M category 3 model, C_4M category 4 model

Model	Training		Full cross-validation	
	R^2	RMSE	R^2	RMSE
GM	0.63	5,652	0.53	6,597
C_1M	0.86	1,391	0.53	2,741
C_2M	0.94	328	0.19	1,499
C_3M	0.97	498	0.84	1,464
C_4M	0.99	289	0.64	2,251

Morton et al. 2011; García-Mozo et al. 2010). Also some studies have focussed specifically on the olive tree, seeking in most cases to predict flowering onset and pollination intensity using linear regression techniques (Galán et al. 2001a; Ribeiro et al. 2006a)

Only a few authors (e.g. Reynolds et al. 2002; Yu et al. 2010) have applied PLSR to phenological research, but this technique has never been used in aerobiological studies. The most novel aspect of this study was that it differentiated between four different year categories using a year clustering approach prior to constructing the regression model. Clustering has been used previously in phenological and aerobiological research, not with the aim of predicting specific features of flowering intensity, but rather for general short- or long-term forecasting purposes (Sánchez-Mesa et al. 2002, 2005). The ANN model provided the most accurate classification of years into groups. Other authors have compared the effectiveness of discriminant analysis and neuronal network analysis for classifying years with different pollen intensities, and to make other predictions regarding pollen season characteristics. (Aznarte et al. 2007; Kasprzyk et al. 2011; Voukantsis et al. 2010; Rodríguez-Rajo et al. 2010; Sánchez-Mesa et al. 2005; Puc 2012).

The physiological response of plants to the same environmental stimuli may differ depending on the weather conditions in any given year (Galán et al. 2001b). Year-clustering took into account both weather-related variables and phenological variables to determine the potential physiological status of the olives in the study area. Four year-categories were generated, each comprising years sharing similar weather conditions, giving rise to similar phenological characteristics. Specific models to predict flowering intensity were generated for each year category. Though minor differences were apparent in each category model (CM), analysis of inter-model coefficients of variation revealed more marked differences.

The general model (GM), constructed using data for all 30 study years, showed that rainfall in February and March exerted a major influence on flowering intensity, expressed as PI; similar findings have been reported by other authors (Recio et al. 1996; Galán et al. 2001a). The GM also indicated that low rainfall and higher minimum temperatures in summer led to increased flowering intensity. The amount of pollen available for spring flowering is influenced by weather conditions over the previous summer, by which time the mother cells designated to become pollen grains are already present. When the summer is characterised by abnormally high temperatures and low rainfall, high pollen counts tend to be recorded during the following spring (Mandrioli 1987). In the olive, the rate of flowering-bud differentiation is affected strongly by the prevailing weather conditions over the previous summer (Rallo and Cuevas 2004), which could prompt a higher rate of fruit abortion, which would increase floral induction (Dag et al. 2010).

The major influence of March–April temperatures has also been reported in other research carried out in the Mediterranean area. A number of authors have found that mild weather, i.e. narrow temperature ranges and high minimum temperatures are associated with greater flowering intensity

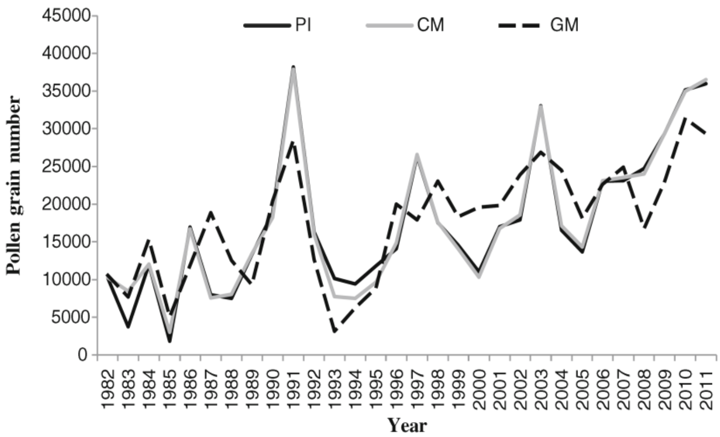

Fig. 2 Observed pollen index (*PI*), and pollen indices predicted by category model (*CM*) and general model (*GM*)

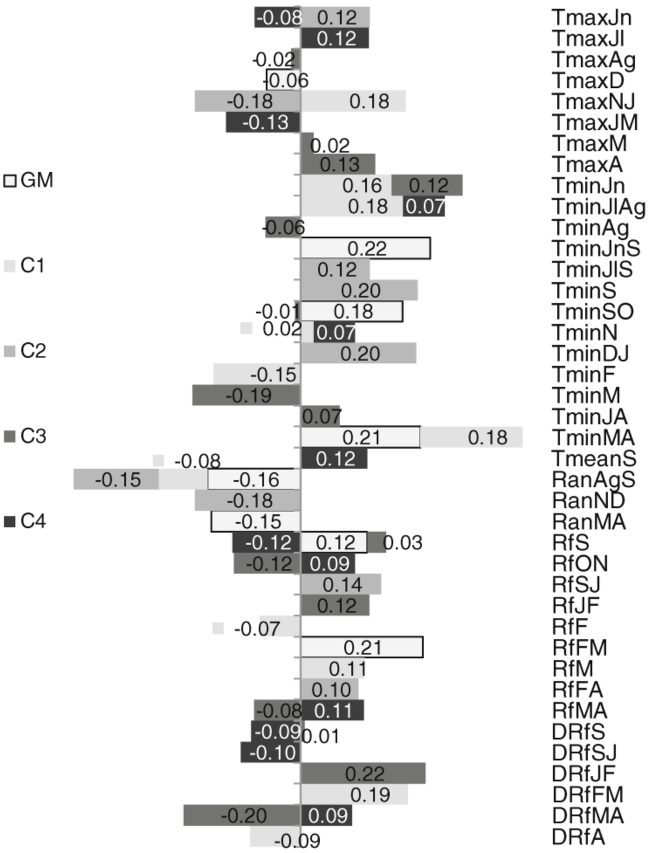

Fig. 3 Coefficients of important variables. The variables were defined prior to building the models—the coefficients show the relative importance of each variable on each model. Model variables: *Tmax* Average value of daily max temperatures; *Tmin* average value of daily minimum temperatures; *Tmean* average values of daily minimum and max temperatures; *Ran* average values of temperature range; *Rf* sum of total rainfall, *DRf* number of days with rainfall above 1 mm, both in individual months (single letters indicating month, e.g. *J* January, *F* February, *M* March…) and in different periods: *JnS* 1 June–30 September, *JlAg* 1 July–31 August, *JlS* 1 July–30 September, *SO* 1 September–31 October, *SJ* 1 September–31 January, *ON* 1 October–30 November, *ND* 1 November–31 December, *NJ* 1 November–31 January, *DJ* 1 December–31 January, *JF* 1 January–28 February, *JM* 1 January–31 March, *JA* 1 January–30 April, *FM* 1 February–31 March, *FA* 1 February–30 April, *MA* 1 March–30 April

(Galán et al. 2001a). The GM displayed a strongly negative coefficient for minimum temperatures in September and October, probably because this early cold impairs bud development. However, lower temperatures in winter, and particularly in December, are reported to favour flowering (Ribeiro et al. 2006b; Orlandi et al. 2010). Analysis of the optimal weather conditions for olive flowering suggests that these tend to occur in the Mediterranean climate, which would account for the high degree of adaptation of the olive in the study area.

Cluster analysis showed that C1 years were marked by dry springs and winters, with cooler temperatures throughout the year; flowering intensity was very poor. Temperature ranges in the summer have a negative effect on flowering intensity, possibly because in summers with below-average mean temperatures, a higher temperature range means that the minimum temperatures are even lower.

C2 years were also characterised by cold winters and springs, but did not display the low rainfall typical of C1 years. Flowering intensity was also poor. In the C2 years, winter temperatures had a more intense effect than in general: minimum temperatures in December to January had a more positive effect, while the wide temperature range in November and December exerted a negative influence; this behaviour can be understood in a context of cold years.

C3 years were marked by warm summers, winters and springs. Pollen counts were above the average for Cordoba, and the pollen season started earlier than in other year categories. Precipitation was lower than average in C3 years, and this could explain why rainfall in January and February exerted a positive effect on the flowering intensity, whereas rainfall from October to November and from March to April showed a negative effect. On the other hand, an influence of March temperatures was noted; maximum temperatures exerted little influence, while high minimum temperatures in March had a significant negative effect, perhaps because they were particularly high in C3 years.

C4 comprised years with hot summers and mild autumns, and heavy rainfall in both seasons. Weather conditions were most conducive to high flowering intensity, as evident in high PIs, high pollen peaks and high daily pollen counts. The category-specific model indicated that summer temperatures exerted a positive effect on PI, perhaps because only extremely high summer temperatures can produce a significant fruit abortion that can affect the next spring flowering. Spring and autumn rainfall also had a positive influence, although in the context of very wet years; September rainfall did not have any positive influence.

The biological responses of plants to climatic variables are rather complex. As stated above, the same weather conditions can act differently on different plants depending on their physiological status but plants are also highly influenced by adaptation to geographical features and local climates (García-Mozo et al. 2009). The models obtained in this study are derived from an empirical approach in a specific Mediterranean area and for these reasons should be applied to different regions with extreme caution. Although the methodology developed in the present work could be considered applicable in other areas, the proposed models were built for a specific region, and therefore are not strictly transferable to different locations.

In overall terms, specific cluster-based CMs proved more effective than the general model. Category differentiation enhanced the effectiveness of phenological modelling for two main reasons:

1. Weather conditions act differently on each year category, since plant physiological status at any given time varies from one category to another.

2. The years comprising each category share similar flowering intensity characteristics, so forecasting models are subject to less error.

These category-specific models proved to be more effective than general models, and therefore better suited to the variability of the Mediterranean climate, probably because plants respond differently to the same environmental stimuli depending on the weather conditions in any given year. Moreover, analysis of the influence of weather patterns on olive phenology will help us to understand the short-term effects of climate change on olive crop in the Mediterranean area that is highly affected by it.

Conclusions

Predictive models obtained using clustering analysis are more effective than general models because the years comprising each category share similar aerobiological characteristics and because weather conditions can act differently on flowering intensity depending on previous meteorological context that could have been decisive for configuring the physiological status of plant.

PLSR proved valuable for generating phenological forecasting models.

The autoregressive and biometeorological indices used to take into account the effects of extreme weather events yielded optimal results in the construction of an effective classification model. ANNs proved a useful tool for effective classification.

We corroborated the finding that summer weather conditions play a major role in olive flowering intensity.

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6. CAPÍTULO III

Modelling olive phenological response to weather and topography

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Modelling olive phenological response to weather and topography

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ABSTRACT

A detailed analysis was made of the response of olive floral phenology to climate and topography in southern Spain. Field phenological, topographical and meteorological data collected at 12 sampling sites in the province of Córdoba over a 17-year period (1996–2012) were statistically analyzed and used to model local olive phenological behaviour.

The study sought to determine: (1) the optimal frequency of phenological sampling during the reproductive period; (2) the major topographical parameters governing local olive reproductive phenology; and (3) the most influential meteorological variables. Findings for the Sign test indicated that weekly sampling yielded accurate results. Correlation and multiple linear regression analysis revealed that altitude and percentage eastward slope were the most influential topographical factors; a positive correlation was detected between delays in phenophases onset and increased altitude and eastward orientation. Correlation and partial least square regression analysis identified air temperature, rainfall, crop evapotranspiration and solar radiation as the major weather factors influencing local olive phenology.

1. Introduction

Phenology was initially defined as "the study of the timing of recurring events, the causes of their timing with regard to biotic and abiotic forces, and the interrelation among phases of the same or different species" (Lieth, 1974). More recent definitions, however, stress the important influence of the environment; Schwartz (1999), for example, notes that "phenology includes the study of periodic events as influenced by the environment, especially temperature changes driven by weather and climate".

Plant reproductive phenology is heavily dependent on environmental conditions. Close examination of phenological behaviour, and of the bioclimatic factors influencing it, is of particular value in areas that are theoretically affected by climate warming, such as the Mediterranean region. This is especially true for major crops such as the olive, *Olea europaea* L., which is grown today not only over much of the Mediterranean basin, but also in south-western Asia and in the Americas, including California, Chile and Argentina (Barranco et al., 2008). The olive is clearly a key crop in agricultural, socioeconomic and environmental terms (Lavee, 1996). It is also important for health reasons the consumption of olive oil is seems to be regarded as beneficial (Tuck and Hayball, 2002), while olive pollen is associated with widespread allergic reactions (D'Amato et al., 2007; Barber et al., 2008). Andalusia region (southern Spain) is the world's largest olive-producing area, accounting for 61% of the total planted to olives in Spain; olive groves are concentrated mainly in provinces such as Jaen and Córdoba (Andalusia Statistical Yearbook, 2011).

Annual olive vegetative and reproductive cycles – and therefore annual fruit yield, which is directly related to flowering intensity (Fornaciari et al., 2005; Galán et al., 2008; García-Mozo et al., 2008; Orlandi et al., 2010a) – seems to be strongly influenced by environmental conditions. The complex phenological response to milder winter temperatures provides a reliable bio-indicator of the impact of climate change in a number of plant species (Osborne et al., 2000; Lambs et al., 2006; Menzel et al., 2006; García-Mozo et al., 2010; Gunderson et al., 2010; Xiao et al., 2013). The present study sought to examine the response of olive crops in the province of Córdoba to weather-related and topographical variables, using field phenological, topographical and meteorological data collected over a 17-year period from 1996 to 2012. Bud break in this area starts in around March, and the main flowering period is usually recorded in May, although changing weather conditions often prompt variations in timing, which have become particularly apparent over recent decades (Galán et al., 2005; Bonofiglio et al., 2008; García-Mozo et al., 2010; Orlandi et al., 2013a).

Although a combination of photoperiod and temperature determines the olive flowering period, it has been shown that temperature largely controls the reproductive development of the olive

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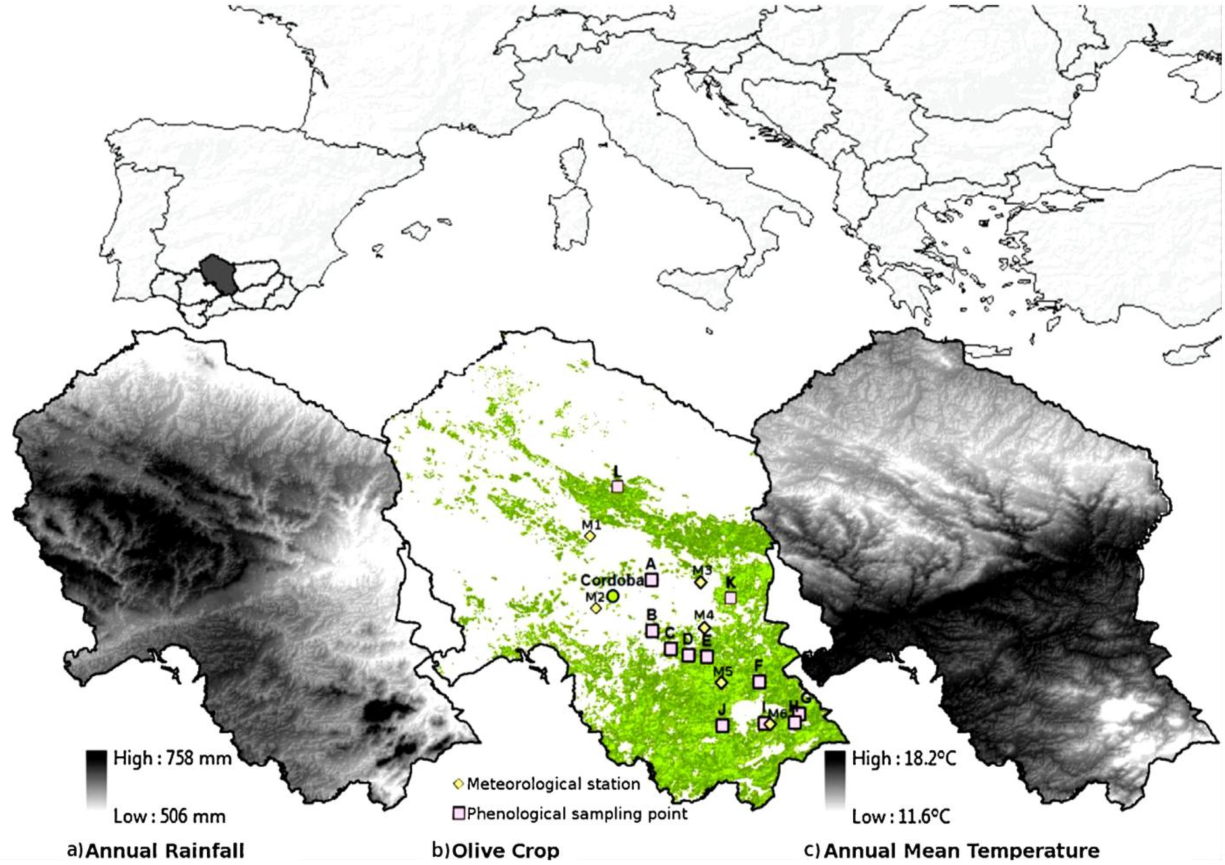

Fig. 1. Location of Córdoba province. (a) Annual rainfall distribution in Córdoba province. (b) Olive crop distribution, location of sampling sites (A–L) and weather stations (M1–M6). (c) Annual mean temperature distribution.

tree, especially during pre-flowering and anthesis (Galán et al., 2001, 2005; Melo-Abreu et al., 2004; García-Mozo et al., 2009; Sicard et al., 2012). Floral induction takes place during the previous summer, and a chilling period is required in order for buds to break winter dormancy (Rallo and Martin, 1991; Orlandi et al., 2006; Andreini et al., 2008). Subsequently, a warm period is required in order for the plant to accumulate sufficient forcing units physiological heat to enable flower development (Galán et al., 2005; Orlandi et al., 2010b). Logically, topography also plays an important role in phenological development, which varies as a function of different photoperiods and weather conditions (García-Mozo et al., 2006; Aguilera and Ruíz-Valenzuela, 2009).

The phenological characteristics of any area are dependent on local climate, which is in turn governed both by macroclimatic conditions, defined by variables such as latitude, and by determinants of microclimatic conditions such as topography. Within a reduced study area, knowledge of microclimatic conditions is essential for a full understanding of differences in reproductive phenology. In order to identify the variables responsible for variations in phenotypic expression in the province of Córdoba, an analysis was made of the correlations between several topographical and weather-related variables and the specific phenological features of various sampling sites.

The main goal of the study was to model olive floral phenological behaviour, taking into account variations in the main factors influencing that behaviour at different sites in the province of Córdoba (Spain) over the last 17 years. Specifically, the study sought to determine: (1) the optimal frequency of phenological sampling during the reproductive period; (2) the major topographical variables governing local olive reproductive phenology; and (3) the most influential weather-related variables.

2. Materials and methods

2.1. Area of study and weather data

The study was carried out in the province of Córdoba, Andalusia (southern Spain) (Fig. 1). The province is located in the Mediterranean region. Local vegetation and crops are adapted to drought periods that last between two and nine months every year. Córdoba city is located in the valley of the Guadalquivir river. The annual mean temperature is 17.8 °C and the annual average rainfall is 621 mm; weather conditions vary greatly year-on-year. Moreover, the continental effect is reflected in particular thermal and rainfall regimes: low rainfall, low relative humidity, and wide daily and annual temperature ranges, are characteristic of the study area (Domínguez-Bascón, 2002). Torrential rains usually occur. Peak daily temperatures are recorded in the afternoon and minimum temperatures at dawn.

The study area, the olive crop distribution, and the location of sampling sites and weather stations, are shown in Fig. 1. This figure also shows variations in annual mean temperature and rainfall within the province. The characteristics of sampling sites are shown in Table 1. The main olive cultivar in the Cordoba province is "Hojiblanca", except for some minor areas in the south of the province, with moreover "Hojiblanca", "Picudo" cultivar is also present and in which are located some of our sampling points (namely Cabra, Carcabuey, Priego and Fuente Tójar).

Four topographical variables were analyzed for each site: altitude (m), maximum slope inclination (%) and maximum slope orientation (%) from South to North and from East to West. Slope orientation ranged from 0% to 100%; values close to 100% indicated maximum South and East orientation, while values close to 0% indicated North and West orientation.

Table 1
Characteristics of sampling sites.

Sites	Code	Years	Altitude (m)	Coordinates
Alcolea	A	2002–2012	160	37°55′ N, 4°40′ W
Torres Cabrera	B	2003–2012	157	37°45′ N, 4°40′ W
Santa Cruz	C	1996–2012	165	37°42′ N, 4°36′ W
Espejo	D	2003–2012	360	37°40′ N, 4°32′ W
Castro del Río	E	1996–2012	261	37°40′ N, 4°28′ W
Baena	F	1996–2012	450	37°35′ N, 4°18′ W
Fuente Tójar	G	2003–2012	567	37°29′ N, 4°10′ W
Priego de Córdoba	H	2003–2012	535	37°28′ N, 4°11′ W
Carcabuey	I	2003–2012	563	37°28′ N, 4°17′ W
Cabra	J	2003–2012	482	37°27′ N, 4°25′ W
Bujalance	K	1996–2012	222	37°54′ N, 4°23′ W
Pozoblanco	L	1996–2012	649	38°23′ N, 4°51′ W

Data were obtained from the weather stations nearest to sampling sites, designated M1–M6 (Fig. 1). All weather stations belong to the Andalusian Phytosanitary Information Alert Network (RAIF; www.juntadeandalucia.es) and the Spanish Meteorological Agency (AEMET; www.aemet.es). Geo-meteorological data were supplied by Worldclim database (Hijmans et al., 2005; www.worldclim.org). The following weather-related parameters were included in the analysis: average maximum and minimum temperature, cumulative rainfall, Chilling Units (CU), Growing Degree Days (GDD), Potential Evapotranspiration for the olive crop (ETc), cumulated rainfall minus ETc (Rf-ETc), and net Radiation (Rn) from January to June, because this time cover all the flowering development period. Bi-monthly, monthly, fortnightly and 10-day data were used, with a total of 288 variables. Chilling Units were calculated following the Utah method (Richardson et al., 1974). In view of local weather conditions, the chilling period was considered as the period from 1 November to 31 January, as is the date in which change the yearly thermal trend (Orlandi et al., 2004; Aguilera et al., 2013).

Heat requirements were calculated as "Growing Degree Days" (GDD°). In the light of earlier findings for this study area, 12.5 °C was used as threshold temperature calculated in previous studies as start limit for heat accumulation (Alcalá and Barranco, 1992; Galán et al., 2001). The sine wave method was used to calculate GDD° (Snyder, 1985). The first day for starting heat accumulation is considered 1 February, when the chilling accumulation period is finished.

Evapotranspiration for the olive crop was calculated using the Penman-Monteith formula, as corrected by the FAO (Allen et al., 1998). This formula includes the following daily parameters: maximum and minimum air temperature, mean air temperature, solar radiation, wind speed, maximum and minimum humidity. The recommended FAO crop rates were used (Pastor and Orgaz, 1994).

2.2. Phenological data

We also analyzed field phenology at 12 different sites: Alcolea at 160 m a.s.l., Torres Cabrera at 157 m a.s.l., Santa Cruz at 165 m a.s.l., Espejo at 360 m a.s.l., Castro del Río at 261 m a.s.l., Baena at 450 m a.s.l., Fuente Tójar at 567 m a.s.l., Priego de Córdoba at 535 m a.s.l., Carcabuey at 563 m a.s.l., Cabra at 482 m a.s.l., Bujalance at 222 m a.s.l. and Pozoblanco at 649 m a.s.l. (Table 1 and Fig. 1). Ten olive trees were periodically observed in all sites, from dormancy to the start of fruit development. The standardized BBCH scale was used to monitor phenological growth (Zadoks et al., 1974; Sanz-Cortés et al., 2002). The onset of each phenophase was designated by the relevant Julian day number, and the number of days was indicated in cases of phenological amplitude.

The following 10 phenophases were analyzed:

50: Inflorescence buds in leaf axils completely closed.
51: Inflorescence buds starting to swell on stem.
52: Inflorescence buds open.
54: Flower clusters growing.
57: The corolla, green-coloured, is longer than the calyx.
61: Beginning of flowering: 10% of flowers open.
65: Full flowering: at least 50% of flowers open.
67: First petals falling.
68: Majority of petals fallen or faded.
69: End of flowering, fruit set, non-fertilized ovaries fallen.

Three intervals of phenological amplitude were also analyzed:

Pre-flowering amplitude (51–61).
Flowering amplitude (61–67).
Post-flowering amplitude (67–68).

2.3. Statistical analysis

2.3.1. Optimal frequency of phenological sampling

The optimal frequency of field sampling was determined by statistical comparison of actual daily phenology data with daily estimates based on from weekly, twice-weekly and fortnightly data at the Alcolea site in 2002, the pilot sampling point. Linear interpolation was used to calculate estimated data. Differences between estimated and real data were checked using the Sign test, due to non-normal data distribution (Hodges, 1955). The SPSS 8.0 software package was used.

2.3.2. Phenology and topography

Relationship between phenological features and topographical factors at each site was analyzed. The study period between 2003 and 2012 was used for this purpose, as it is the longest time series covering all study sites (Table 1). The average onset of each phenophase was taken as the overall phenological feature for each site. For statistical analysis, every site has been considered separately. The correlation between topographical variables and phenophases was analyzed using two different approaches: (1) Spearman correlation analysis and (2) multiple linear regression (MLR) analysis. The SPSS 8.0 software package was used for this purpose.

2.3.3. Phenology and weather-related variables

For these statistical analyses, values for weather variables in each year and at each site were considered separately. The study period between 1996 and 2012 was used for this purpose. The influence of weather variables on phenological development was analyzed by (1) Spearman correlation analysis as a univariate approach; (2) partial least squares regression (PLSR) analysis as a multivariate approach. PLSR analysis was considered more appropriate than MLR in these kinds of studies, given the large number of predictor variables (288) (Carrascal et al., 2009). The SPSS 8.0 software package was used for correlation analysis and the Unscrambler 9.7 package for PLSR analysis.

Although biological and weather features varied among sites, for PLSR analysis all sites were analyzed together for obtaining more robust results, although this fact can reduce the adjustment of the models. For this purpose, all variables were typified to prevent some sampling sites having more weight than others in the statistical interpretation. The following formula was applied:

$$Z_i = \left(\frac{X_i - \bar{X}}{\sigma}\right) \quad (1)$$

where X_i is an analyzed case of variable X; Z_i is the typified value for X_i; $\bar{X}$ is the average value of variable X; σ is the standard deviation of variable X.

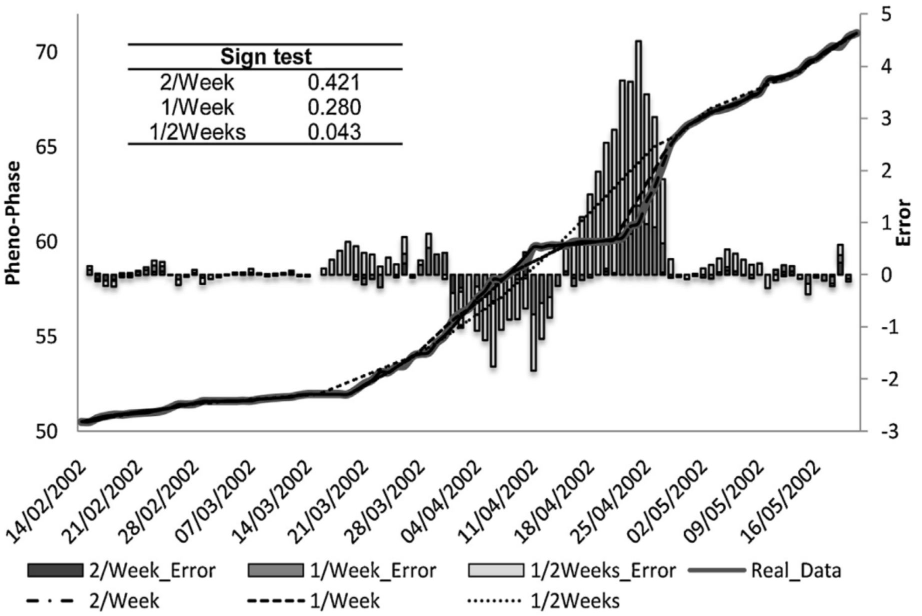

Fig. 2. Actual daily phenological data for the Alcolea site in 2002, compared with data interpolated from twice-weekly (2/week), weekly (1/week) and fortnightly sampling (1/2 weeks). Sign test to errors between actual and interpolated data are shown in the diagram.

3. Results

3.1. Phenological sampling frequency

Actual daily phenological values, together with those obtained by linear interpolation from weekly, twice-weekly and fortnightly data, are shown in Fig. 2. Significance levels using the Sign test are also provided. Although interpolated twice-weekly data displayed the greatest affinity with daily data, there were no significant differences with respect to interpolated weekly data. By contrast, interpolated fortnightly data exhibited significant differences with respect to actual daily data.

Analysis of the error between real and interpolated values (Fig. 2) showed that an increased interval between two samplings prompted greater error, and that greater error was detected in periods of faster phenological development. In the light of these findings, weekly sampling was deemed to be accurate.

3.2. Phenology and topography

Analysis of average phenophase timing at each site (Fig. 3) revealed a progressive delay in onset, as a function of altitude. Correlations between topographical and phenological variables are shown in Table 2. The results of regression analyses to determine the topographical causes of phenological differences detected between sampling sites are shown in Table 3.

Findings suggested that altitude and maximum slope orientation were the main factors responsible for differences in phenophase timing. Correlation and regression analysis confirmed that altitude was the topographical factor most influencing phenological development; a positive correlation was detected between delays in phenophase onset and increased altitude, but not between altitude and the phenophase amplitudes studied. Phenophase onset was also delayed as eastward orientation increased. Moreover, correlation analysis indicated that eastward orientation was associated with shorter flowering periods. Regression analysis confirmed that altitude accounted for over 60% of the variance in phenotypic expression of all phenophases, while percentage eastward slope accounted for over 20%. At some sites, however, topography accounted for over 90% of phenological variability.

Table 2
Spearman correlation coefficient for phenophases and topographical factors. PRE (preflowering period); FLOW (flowering period); POST (postflowering period); ALT (altitude); PEN (maximum slope); EW (percentage of maximum slope orientation to East and West); SN (percentage of maximum slope orientation to South and North).

	ALT	PEN	EW	SN
Phenophase 51	0.891^{**}	0.018	0.642^{*}	−0.207
Phenophase 52	0.869^{**}	0.159	0.791^{**}	−0.187
Phenophase 54	0.939^{**}	0.134	0.679^{*}	−0.226
Phenophase 57	0.927^{**}	0.110	0.734^{*}	−0.159
Phenophase 61	0.903^{**}	0.159	0.697^{*}	−0.116
Phenophase 65	0.939^{**}	0.134	0.679^{*}	−0.226
Phenophase 67	0.939^{**}	0.134	0.679^{*}	−0.226
Phenophase 68	0.927^{**}	0.085	0.697^{*}	−0.116
PRE	0.176	0.256	0.147	0.043
FLOW	−0.245	0.225	-0.688^{*}	−0.548
POST	−0.340	−0.012	−0.055	0.413

* $p < 0.05$.
** $p < 0.01$.

3.3. Phenology and weather-related variables

3.3.1. Correlation analysis

A negative correlation was observed between water availability (Rf-ETc) in late January and the onset of all phenophases of pre-flowering and flowering. Thus, when

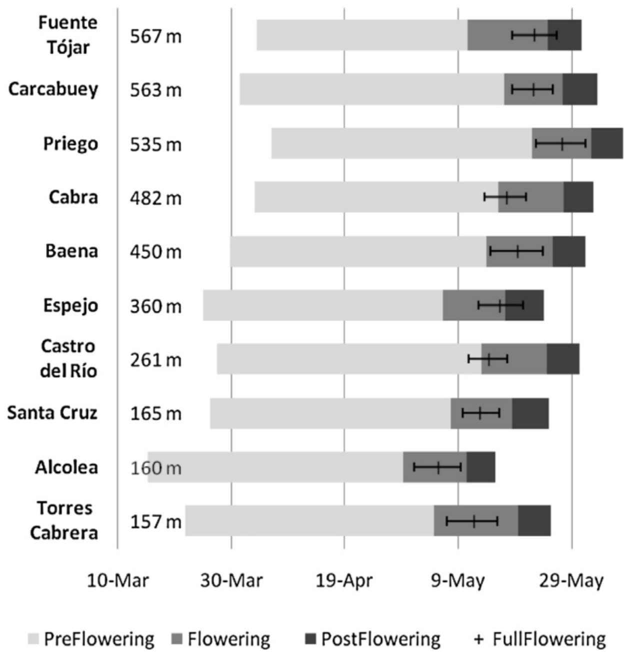

Fig. 3. Average phenological behaviour of each olive grove over the period 2003–2012. Full flowering (average date of phenophase 65 ± 95% confidence limit).

Table 3
Linear relationship between phenological behaviour and topographical data. ALT (altitude); EW (maximum slope orientation to east and west, %).

Phenophase	Formula	Beta coefficient	R^2	p
51	PP51 = 75.527 + 0.028(Alt) + 0.045(EW)	Alt = 0.747EW = 0.228	0.772	0.006
52	PP52 = 82.846 + 0.031(Alt) + 0.109(EW)	Alt = 0.643EW = 0.401	0.823	0.002
54	PP54 = 94.256 + 0.032(Alt) + 0.101(EW)	Alt = 0.679EW = 0.377	0.847	0.001
57	PP57 = 109.215 + 0.0293(Alt) + 0.078(EW)	Alt = 0.678EW = 0.325	0.778	0.005
61	PP61 = 118.650 + 0.028(Alt) + 0.065(EW)	Alt = 0.718EW = 0.300	0.813	0.003
65	PP65 = 125.000 + 0.028(Alt) + 0.053(EW)	Alt = 0.772EW = 0.260	0.856	0.001
67	PP67 = 131.441 + 0.0275(Alt) + 0.048(EW)	Alt = 0.807EW = 0.251	0.908	0.000
68	PP68 = 137.37 + 0.026(Alt) + 0.057(EW)	Alt = 0.770EW = 0.304	0.910	0.000

water was plentiful, the onset of floral bud development (51) was brought forward. Another water-related variable showing a strong influence at all sites was cumulative rainfall during March and April, especially in the pre-flowering period. Higher rainfall in these months increased the rate of phenological development. Net solar radiation in January, especially during the first fortnight, displayed a negative correlation with pre-flowering and flowering phenophase onset at all sites. Net solar radiation recorded in May displayed a strong negative correlation with the duration of post-flowering.

Air temperature was found to be the main factor influencing olive floral phenology. Maximum, minimum and mean air temperatures during flowering exhibited a strong negative correlation with the onset and duration of all phenophases at all sites. Higher temperatures led to advanced onset dates and shorter phenophase duration. Minimum temperatures in March exerted the greatest influence on the duration of both pre-flowering and flowering periods. Flower development phenophases, closer to full flowering (54 and 57) were especially influenced by temperatures in late February, March and early April, a strong negative correlation being observed with both maximum and minimum air temperatures. These variables, and particularly minimum air temperatures in March and April, displayed a strong negative correlation with the onset of flowering and the date of peak flowering (61 and 65). The heat-related variable most affecting post-flowering phenophases (67 and 68) was maximum temperature in May, especially in Baena (located at a higher altitude).

The number of Chilling Units accumulated during November, December and January correlated negatively with all phenophases, i.e. greater accumulation of cold prompted faster phenological development. The GDD° accumulated by the onset of flowering also displayed a strong negative correlation with the duration of flowering and post-flowering. A greater accumulation of GDD° led to faster phenological development and earlier flowering dates.

3.3.2. PLSR analysis

In order to determine which weather variables influenced phenological development and ascertain the magnitude of their effects, PLSR analysis was applied to the main phenological dates: onset of floral bud development (51), onset of flowering (61) and maximum flowering date (65). For phenophase 51, the resulting model included 2 principal components which jointly accounted for 92% of the variance shown by independent variables (R^2X) and 88% of the variance displayed by the dependent variable (R^2Y); the full cross-validation Q^2 was 0.77. For onset of flowering the model also included 2 principal components, jointly accounting for 78% and 83%, respectively, of the variance displayed by independent variables (R^2X) and the dependent variable (R^2Y); full cross-validation Q^2 was 0.71. Finally, for flowering the model accounted for 84% and 75%, respectively, of the variance exhibited by the independent variables (R^2X) and the dependent variable (R^2Y); full cross-validation Q^2 was 0.52.

As Fig. 4 shows, water availability at the beginning of the year (i.e. accumulated rainfall minus accumulated evapotranspiration, from 1 October to 31 January) had a marked influence on all phenophases, and especially on phenophase 51. Similarly, accumulated rainfall in March and April prompted an earlier flowering onset.

The variables most influencing phenological development were heat-related. An increase in both accumulated Chilling Units from 1 November to 31 January, and Growing Degrees Days from 31 January to 31 April prompted faster phenological development. The temperature range between January and April exerted an effect similar to that of heat accumulation. Average temperatures in February and minimum temperatures in March had a particularly strong influence on the onset of phenophase 51.

4. Discussion

Findings on floral phenology provide important information regarding the reproductive biology of crop species. A number of studies have examined olive floral development in a range of climatic areas (Melo-Abreu et al., 2004; Ribeiro et al., 2006; Tormo et al., 2011; Dimou, 2012). While most report a correlation between phenological development and weather-related variables (Avolio et al., 2008; Bonofiglio et al., 2009), others additionally note links with topographical features (García-Mozo et al., 2006; Aguilera and Ruíz-Valenzuela, 2009). The variety grown and the agricultural management regime used are also known to influence olive phenology (García-Mozo et al., 2009; Bacelar et al., 2009; Dias et al., 2012).

The present study provided statistical confirmation that the use of an average phenological stage representing each olive grove yields reliable results, as does the linear interpolation of weekly data in order to estimate daily phenological data. In view of the cost-benefit balance, weekly sampling would appear to be the best option.

Analysis of correlations between phenological development and topographical factors revealed that altitude was the single most influential variable, since at higher sampling sites the weather is colder. Similar findings are reported by numerous authors (Fornaciari et al., 2000; García-Mozo et al., 2006; Aguilera and Ruíz-Valenzuela, 2009). Delayed onset of phenophases was observed with increasing eastward orientation, perhaps because this determines the amount and quality of light reaching the plants. The olive variety grown is another key determinant of phenological behaviour; however, since "Hojiblanca" is by far the most widely grown cultivar in the study area, this had little effect on overall results.

The findings of this study confirm that air temperature is the factor most affecting phenophase onset and duration (Galán et al., 2005; Orlandi et al., 2013b). Weather conditions may have varying effects on the olive tree reproductive cycle, depending on the previous meteorological context, which might have a decisive influence on the plant's physiological status (Oteros et al., 2013a). This is borne out by analyses revealing that the same weather-related variables displayed different correlations with phenophase onset and duration at different sites. However, phenophases 54 and 57

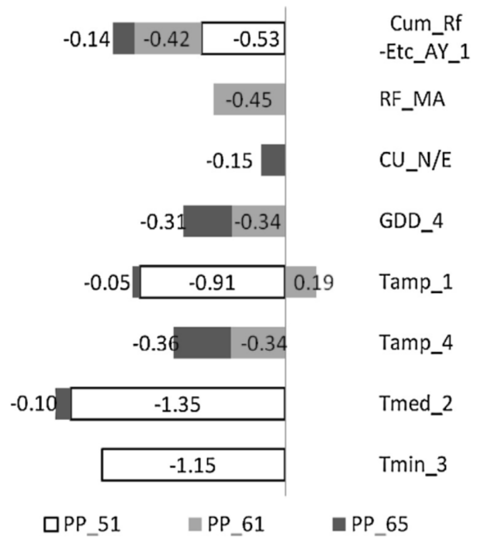

Fig. 4. Coefficients of important variables for onset of phenophases 51, 61 and 65.

(closer to flowering) were the most affected, probably because during this period the plant requires more energy as is related to pollen formation (Mert et al., 2013).

Water availability during the earlier floral phenophases (31 January) proved to be a major variable for all phenophases between 51 and 65. Here, limited water availability affected olive tree development at various phenological stages (Rapoport et al., 2012; Moorthy et al., 2011; Oteros et al., 2013b).

As also reported by Orlandi et al. (2004), the present findings highlighted the importance of the number of cold units accumulated between 1 November and 31 January, and of heat units accumulated between the end of the cold accumulation period and the onset of phenophase 51 or the start of flowering.

5. Conclusions

Statistical analysis to determine the optimal field phenological sampling periodicity reveals that weekly field monitoring can be recommended as the minimum acceptable time for future studies on olive reproductive phenology.

The topographical variables most influencing olive reproductive phenology were altitude and East–West orientation of maximum slope, while the most influential weather-related variables were temperature in winter and early spring and water availability for flowering.

Our results covering a wide area and a long time period will be useful for various purposes. The detailed knowledge of the influence of bioclimatical conditions determining phenological behaviour of *O. europaea* is valuable information to model the potential effects of climate change on olive tree, and to estimate the future adaptation of olive growing requirements in different geographical areas.

This response of the olive tree to climate change can have agricultural, economic, social, and public-health effects (allergy); advanced knowledge of its phenological behaviour will not only help us to better understand the reproductive biology of this specie but also to more accurately forecast crop yields.

The present findings should be borne in mind when reviewing measures to modify the olive agroecosystem in southern Europe.

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7. CAPÍTULO IV

Better prediction of Mediterranean olive production using pollen-based models

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RESEARCH ARTICLE

Better prediction of Mediterranean olive production using pollen-based models

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Abstract Olive oil is a major economic resource of the Mediterranean region. Olive crop management can be improved by models that forecast the variable reproductive biology of olive tree. However, the processes controlling olive harvest are complex on large scales. Here, we study the parameters that influence olive fruit production for developing accurate forecasting models. Seventeen aerobiological sampling points have monitored olive pollen grains in Spain, Italy and Tunisia from 1993 to 2012. Six crop models have been developed at two provinces and country scales. The modelling has been done in two steps: (1) typification and (2) modelling by partial least square regression. Results show that higher pollen indexes and water availability during spring are related to an increase of final fruit production in all the studied area. Higher pollen indexes are also positively correlated with air temperature during early spring and autumn. Furthermore, a decrease of fruit production is related with increasing air temperature during winter and summer. To conclude, we have designed accurate models that allow accurate predictions of olive production.

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1 Introduction

Olive (*Olea europaea* L.) is one of the most extensive crops in the Mediterranean basin (Fig. 1). Olive fruit and oil are among the most important products for the economy of this area (Lavee 1996). Indeed, 95 % of the total area under olive production is concentrated in the Mediterranean basin. Olive cultivation is believed to have originated about 6,000 years ago in the Middle East (now Israel). The profitability of the cultivation of olive is mainly obtained from olive oil production (90 % of the economic benefit), and hence less so from the commerce of fresh fruit (Vossen 2007; Barranco et al. 2008).

Early and effective crop forecasting is essential for optimisation of technical and human resources for olive harvesting and for planning global marketing and commercial distribution of olive oil. Nowadays, crop fruit production has been forecasted using several methods, such as crop growth monitoring system models, time trends or based on satellite measurements (Palm 1995; Bastiaanssen and Ali 2003; Fei et al. 2012). While the most widely used method for crop estimation is by visual observation prior to the harvest, in the case of

Fig. 1 Olive grove in the South of Córdoba province, where it is the main economical resource. The present paper will improve the productivity of the olive grove in the Mediterranean area

anemophilous species, good results can be obtained by modelling pollen release as an index of the flowering intensity and as a bioindicator of the pollination period (Muñoz et al. 2000; García-Mozo et al. 2012). In anemophilous species, the annual airborne pollen has been demonstrated to be a good indicator of flowering intensity and timing during certain phenological stages (Keynan et al. 1989; Aguilera and Ruiz-Valenzuela 2009; León-Ruiz et al. 2011). Nevertheless, there are other external parameters that also affect different essential processes on fruit production including in particular the weather, but also pollination, fruit setting, fruit fattening and the favourability for pests. Therefore, different studies on olive production have included meteorological variables in combination with annual airborne pollen to obtain more accurate indications (Galán et al. 2008; Orlandi et al. 2010).

The present study has been designed to identify the main abiotic parameters that influences more olive fruit production in three of the main olive-producing countries of the world: Spain, Italy and Tunisia and also to develop accurate forecasting models of crop production in several areas of the Mediterranean basin. In general, the areas devoted to this crop in those countries have a Mediterranean climate, which is characterised by hot, dry summers and mild, rainy winters. However, this climate area is also characterised by marked year-on-year variations in the weather patterns with great spatial variability. On the other hand, the Mediterranean basin has been identified as one of the most affected areas of the world in future climate change projections. Knowledge about the abiotic parameters that contribute more to crop production in this area can offer important information to predict the potential impacts of climate change (IPCC WGII 2007; Giorgi and Lionello 2008; Lavalle et al. 2009; Sicard et al. 2012; Orlandi et al. 2013).

One objective of this paper has been to propose three integrated national models for olive fruit production in the Mediterranean basin at country scale for Spain, Italy and Tunisia taking into account both different meteorological and airborne pollen parameters for modelling with a novel statistical methodology. A second goal has been focus to construct forecasting models at province scale to provide accurate models that can be better adapted to different crops, cultivars or weather conditions, where different abiotic parameters can play an important role.

2 Materials and methods

Different variables were taken into account in this study, including airborne olive pollen counts, meteorological parameters and agronomic reports over the last 20 years (1993–2012). The studied areas stretch across the Mediterranean basin and included three of the main olive oil production countries in the world (up to 66.5 % of world production): Spain (47 %), Italy (14.5 %) and Tunisia (5 %) (IOC 2011).

In these countries, the main olive-growing areas have been airborne pollen monitored. In Spain, these sampling points cover 74 % of the national production: Jaen (41 %), Córdoba (20 %), Granada (8 %) and Malaga (5 %) (Ssya 2011). In Italy, these sampling points cover 83.2 % of the national production: Umbria (1 %), two in Campania (8 %), two in Puglia (35 %), one in Calabria (29 %) and two in Sicily (10 %) (ISTAT 2011). In Tunisia, these sampling points cover 97 % of the national production: Mornag (23 %), Sahel (22 %), Menzel M'Hiri (20 %), Sfax (25 %) and Medenine (7 %) (GDAP 2011).

The studied areas are with different olive cultivars. In the Spanish study area, the principal cultivars are Hojiblanca, Picual, Picudo and Lechín. In Italy, the cultivars in Sicily are Biancolilla and Nocellara; in other areas, the more important cultivars are Frantoio, Carolea and Coratina. In the Tunisian studied areas, the principal cultivars are Chetoui and Chemlali.

The airborne pollen counts represent the total amount of *Olea* pollen in the air during the pollen season; this variable is termed the Pollen Index (PI). The pollen season start date was defined as the first day in which at least a daily average of 1 pollen grain/m^3 of air was reached, with at least five subsequent days with ≥1 pollen grain/m^3 (García-Mozo et al. 2009). The end of the season was the last day on which 1 pollen grain/m^3 was recorded and when five subsequent days showed <1 pollen grain/m^3. In Spain and Italy, airborne pollen were recorded using Hirst-type volumetric traps (Hirst 1952), which were managed according to the standardised rules laid down by the Spanish Aerobiology Network (Galán et al. 2007). In Tunisia, airborne pollen was detected using Cour traps (Cour 1974). The traps have been sited in the administrative capitals of the growing study areas (Fig. 2).

Meteorological data included maximum and minimum daily temperature, daily rainfall, daily solar radiation,

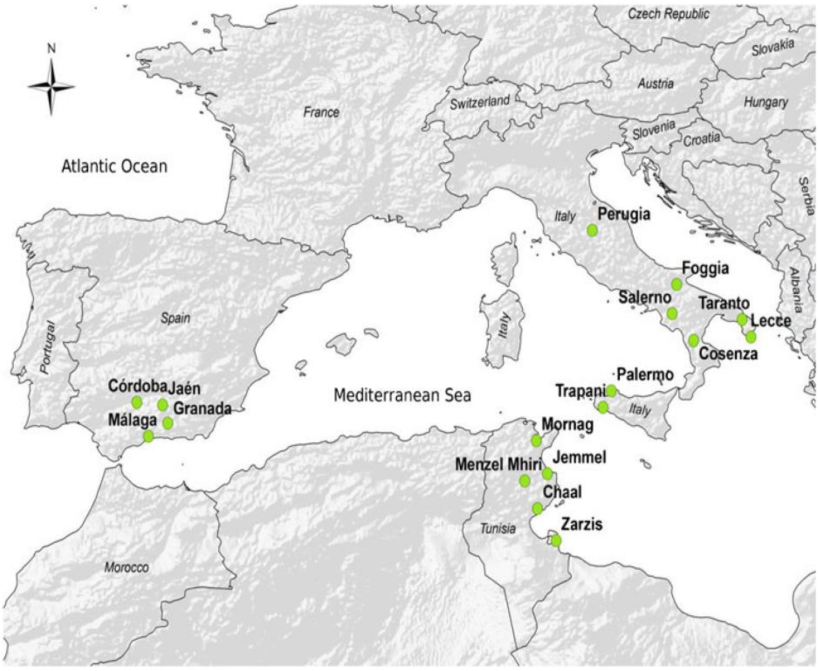

Fig. 2 Locations of the aerobiological sampling points used in this study

maximum and minimum daily relative humidity and mean daily wind velocity. These parameters were recorded at the weather stations nearest to the aerobiological samplers. The meteorological data from Spain were provided by Andalusian Phytosanitary Information Alert Network (RAIF) and the Spanish Meteorological Agency (AEMET). The Italian meteorological data were obtained from the network stations of the National Council of Agricultural Research (CRA-Cma). In Tunisia, meteorological data were provided by the different stations of the National Meteorological Centre (NIM).

The abiotic variables analysed have been mean, maximum and minimum temperature, cumulated rainfall, evapotranspiration of the olive crop (ETc.), cumulated rainfall minus ETc. and net radiation from 1 year before each harvest. These parameters have been included in the analysis as a global, bi-monthly, monthly, fortnightly or 10-day value.

ETc. was calculated following the Penman–Monteith formula as modified by the Food and Agriculture Organization of the United Nations (FAO; Allen et al. 1998). Different daily parameters have been taken into account for ETc. calculation: maximum and minimum air temperature, mean air temperature, solar radiation, wind speed and maximum and minimum relative humidity. For missing meteorological data estimation, the FAO P-M guide proposal (chapter 3) has been followed (Allen et al. 1998). The crop rates proposed by the FAO were used to calculate ETc. (Pastor and Orgaz 1994).

Rainfall and temperature characteristics (Table 1) in each of the study areas share similarities in the Mediterranean climate, although there are some clear differences, i.e. lower annual rainfall and higher temperatures in Tunisia than in Italy and Spain or warmer climate in Spain than in Italy.

In the present study, six models were built based on different area ranges: three national models at country scale for Spain, Italy and Tunisia, and three local models at province scale that represent the central, northern and southern olive growing areas for Cordoba (Spain), Perugia (Italy) and Zarzis (Tunisia), respectively.

Statistical procedure has followed two different steps at both scales; although typification transformation has no effect on the final outcome of the local models:

I. *Typification*. All study sites are with different biological and meteorological features; however, in the case of national models, several areas have been analysed together. For this purpose, all variables were typified before modelling to prevent any particular sampling point with more weight in the statistical interpretation than the others. To avoid these potential errors, the following typification formula has been applied:

$$Z_i = \left(\frac{X_i - \overline{X}}{\sigma}\right) \tag{1}$$

where X_i is an analysed case of variable X, Z_i is the typified value of X_i, $\overline{X}$ is the mean value of the variable X and σ is the standard deviation of variable X.

Table 1 Rainfall and temperature data for each of the aerobiological sampling points, averaged over the period from 1993 to 2012

Growth area	Country	Coordinates	Altitude	Rainfall (mm)				T (°C)			
			m a.s.l.	JFM	AMJ	JlAS	OND	JFM	AMJ	JlAS	OND
Córdoba	Spain	37°50′N, 4°45′W	123	187.4	106.2	37.5	271.6	11.6	20.9	26.9	14.3
Jaén	Spain	37°45′N, 3°47′W	550	163.5	107.6	37.8	179.2	10.4	19.6	25.8	12.9
Granada	Spain	37°11′N, 3°35′W	685	101.5	89.5	60.4	102.3	8.6	18.8	24.7	13.0
Málaga	Spain	36°47′N, 4°19′W	5	189.7	74.8	23.4	254.5	13.5	20.2	25.6	16.3
Perugia	Italy	43°06′N, 12°23′E	450	191.4	200.6	156.1	269.2	6.8	17.7	22.8	10.4
Foggia	Italy	41°25′N, 15°33′E	70	101.5	89.5	60.4	102.3	8.6	18.8	24.7	13.0
Salerno	Italy	40°31′N, 15°22′E	29	302.0	146.3	141.3	299.4	9.2	17.6	21.0	12.4
Taranto	Italy	40°21′N, 18°01′E	48	302.0	146.3	141.3	299.4	9.2	17.6	21.0	12.4
Lecce	Italy	39°49′N, 18°21′E	104	95.4	63.4	73.3	208.7	9.1	18.4	24.3	13.3
Cosenza	Italy	39°43′N, 16°10′E	150	181.4	106.7	106.3	181.2	9.8	19.0	25.1	14.1
Palermo	Italy	38°10′N, 13°05′E	21	71.7	27.5	101.7	166.5	12.5	20.0	25.3	17.5
Trapani	Italy	37°40′N, 12°46′E	160	146.0	54.0	46.7	201.0	11.6	19.0	25.1	16.2
Mornag	Tunisia	36°39′N, 10°16′E	40	158.3	84.4	56.7	173.7	12.5	21.4	27.4	16.9
Jemmel	Tunisia	35°38′N, 10°41′E	30	93.8	48.4	64.3	104.6	13.7	21.4	27.4	18.3
M.Mhiri	Tunisia	35°25′N, 09°50′E	160	61.8	70.6	68.1	79.3	13.7	23.1	29.5	18.1
Chaal	Tunisia	34°34′N, 10°19′E	97	64.6	51.3	35.2	60.7	13.4	21.6	27.4	17.9
Zarzis	Tunisia	33° 35′N, 11°01′E	17	47.0	18.1	25.9	83.7	14.9	23.1	28.0	19.2

JFM January, February, March; *AMJ* April, May, June; *JlAS* July, August, September; *OND* October, November, December

II. *Modelling*. The models were constructed using the partial least squares regression method (PLSr), taking the crop production as a dependent variable and the different meteorological and aerobiological variables as the independent variables. PLSr is considered as an appropriate statistical technique when a high number of observations and a high number of independent variables are involved in the modelling process. Modelling was based on linear transformation of the original descriptors to a small number of orthogonal factors (latent variables) to maximise the covariance between the descriptors and the dependent variables. PLSr method develops latent variables, like the principal component analysis and prior regression, but they are building with the purpose to explain the dependent variable, i.e. the crop production. In this sense, latent variables are built more efficiently than with other multivariate methods. In this paper, each latent variable represented a key factor for olive crop production. All of the models were validated following the full cross validation method. Unscrambler 9.7 software was used.

3 Results and discussion

Some studies have attempted to model the relationship between olive crop production and different airborne pollen and weather variables prior to harvesting (Candau et al. 1998; González-Minero et al. 1998; Fornaciari et al. 2002, 2005; Orlandi et al. 2003, 2005, 2010; Galán et al. 2004, 2008; Ribeiro et al. 2007, 2008; García-Mozo et al. 2008; Aguilera 2012). This paper tries to improve these studies in two points: in all these studies, linear regression approach for solving the problem has been used; in this study, a novel statistical methodology based on two different steps has been used: (1) typification and (2) modelling by PLSr. This statistical analysis allows to model crop production in several areas at the same time. Being the first time that it is modelling more than one province at the same time, the first models, built at country scale, can provide useful information. On the other hand, thanks to the use of a database with the highest study area and study years so far, it is possible to know the main abiotic parameters responsible for crop production at different scales and to obtain global conclusions.

Only the abiotic control on yield and PI has been taken into account due to PI summarising the percentage of variability explained by other area features like the cultivar. Moreover, the most accurate forecasting model has been at the province level; the heterogeneity in biotic features and anthropogenic management at province and national levels precludes its use as crop yield forecasting variables with scientific purposes. For this reason, it is difficult to know its influence on crop production at a large scale. While management features, like the extensive–intensive management or the fertilisation, must be taken into account when the aim is develop accurate crop yield forecasting models at farm scale.

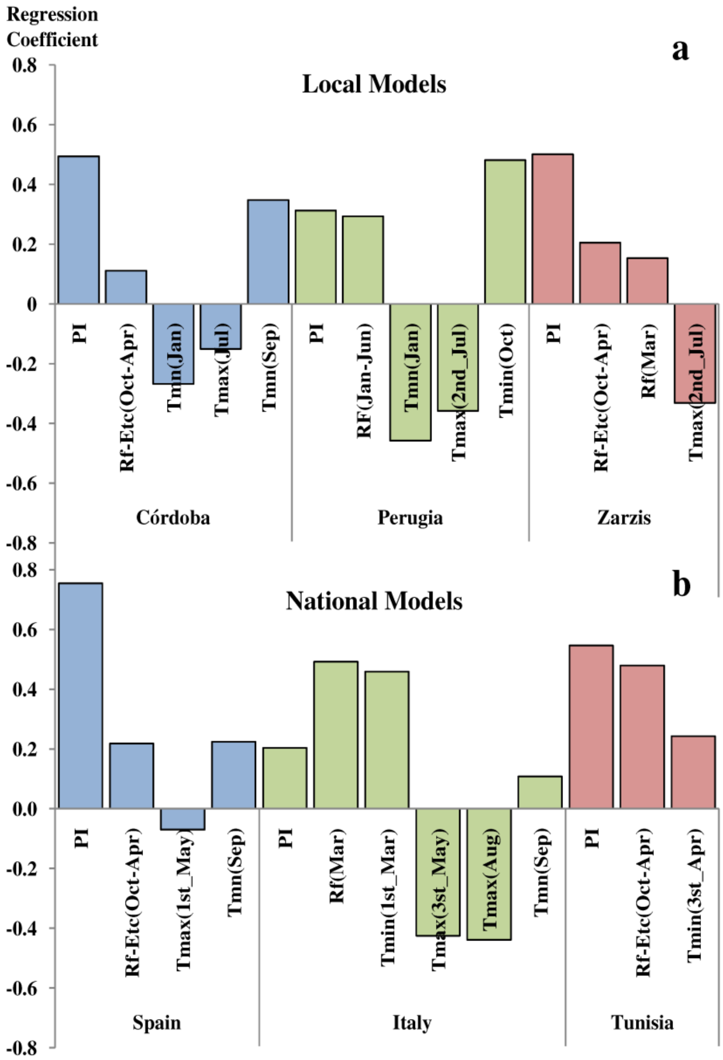

Fig. 3 Regression coefficients of the important variables in local models (**a**) and national models (**b**). *PI* pollen index; *Rf-Etc.(Oct–Apr)* cumulated rainfall from 1 October of the preceding ($n-1$) year to 30 April minus the evapotranspiration for the olive crop over the same period; *Tmn(Jan)* mean January temperature; *Tmax(Jul)* mean daily maximum July temperature; *Tmn(Sep)* mean September temperature; *Rf(Jan–Jun)* cumulated rainfall from 1 January to 30 June; *Tmax(2nd_Jul)* mean daily maximum temperature for the second 10 days of July; *Tmn(Oct)* mean October temperature; *Rf(Mar)* cumulated rainfall from 1 to 31 March; *Tmax(1st_May)* mean daily maximum temperature in the first 10 days of May; *Tmin(1st_Mar)* mean daily minimum temperature in the first 10 days of March; *Tmax(3rd_May)* mean daily maximum temperature in the last 10 days of May; *Tmax(Aug)* mean daily August maximum temperature; *Tmin(3rd_Apr)* mean daily minimum temperature in the last 10 days of April

the number of flowers per inflorescence or provide a greater photosynthetic capacity for the plant (Rallo 1994; Candau et al. 1998; Galán et al. 2004, 2008). Other studies have related the importance of water availability in terms of the process of fruit setting and early fruit drop after fecundation (Galán et al. 2008). Indeed, the presence of a water deficit has important impact on the development of the whole plant, which can be observed at the phenological, physiological and production levels (Bacelar et al. 2007, 2009; Barranco et al. 2008; Rapoport et al. 2012).

Air temperature is another important variable in different areas. However, decoupling from the phenological development time means that different effects can be seen for the various analysed areas. On local models for Cordoba and Perugia, the mean January temperature showed a negative influence on crop production. In late autumn and early winter, olive trees undergo a dormancy period when their growth and development are temporarily suspended (Melo-Abreu et al. 2004). Afterwards, a stress situation due to low temperature is required for them to emerge from dormancy, a chilling period (Orlandi et al. 2004; Galán et al. 2005). Other studies have shown negative relationships between temperatures during this dormancy and harvest production (Fornaciari et al. 2005). High temperatures during this period can delay breaking of dormancy, and therefore delay phenological development (Orlandi et al. 2006).

On the other hand, the minimum temperatures recorded during the pre-flowering period showed a positive relationship for crop production on the national model for Italy (the mean minimum temperatures in the first 10 days of May) and the national model for Tunisia (the mean minimum temperatures in the last 10 days of April). Similar relationships have been observed in other studies, where it has been maintained that exposure of the olive trees to heat before flowering promotes an increase in the photosynthetic capacity and favours the pollination process (García-Mozo et al. 2008).

Furthermore, higher temperatures during fruit setting showed a negative relationship with crop production in the Spanish model (mean maximum temperatures in the first 10 days of May) and in Italy (mean maximum temperatures in the last 10 days of May). The decoupling of the phenological development time will be the reason for the differences in the periods when temperature affects olive in different geographical areas. Other studies have noted that elevated temperatures during the fruit setting period can lead to excessive fruit drop, and hence lower production yield. Although, during this period, a high fruit abortion rate on olive tree, water deficit and high temperature is normal to accentuate this process (Aguilera 2012).

High temperatures and evapotranspiration during the summer are also causes of increased fruit drop, and hence a decline in the crop production, according to various studies (Ribeiro et al. 2008; Orlandi et al. 2010). The present study has also found this relationship for the local models for Córdoba, Perugia and Zarzis, and also in the national model of Italy.

There is also a positive relationship between autumn temperature and crop production in the local models for Córdoba and Perugia and the national models of Spain and Italy. It has been also observed by other studies manifesting the role of these temperatures on the maturation process (Galán et al. 2008).

In general, the dependent variable shows different relationships with the different predictive parameters depending on the studied areas. In this sense, local models offer optimum forecast potential (Fig. 4). However, national models do provide valuable information about the parameters that affect the different study areas more, revealing the most important variables for olive crop production, and which areas will be more fragile in a changing environment.

With regard to the effectiveness of the models in the present study, it can be seen that they were more accurate in local areas. The reason is the heterogeneity when input data increase due to environmental conditions that can influence plants at different study areas. Weather conditions can act in different ways on the reproductive cycle of olive trees depending on the previous meteorological context, which might have been decisive for the physiological status of the plant (Oteros et al. 2013b).

Nevertheless, wider models can still help to understand responses of olive trees in different agroecosystems and to determine the most important variables affecting olive fruit production. The data from these wider models will therefore help to deepen the knowledge of potential effects of climate change in the Mediterranean basin.

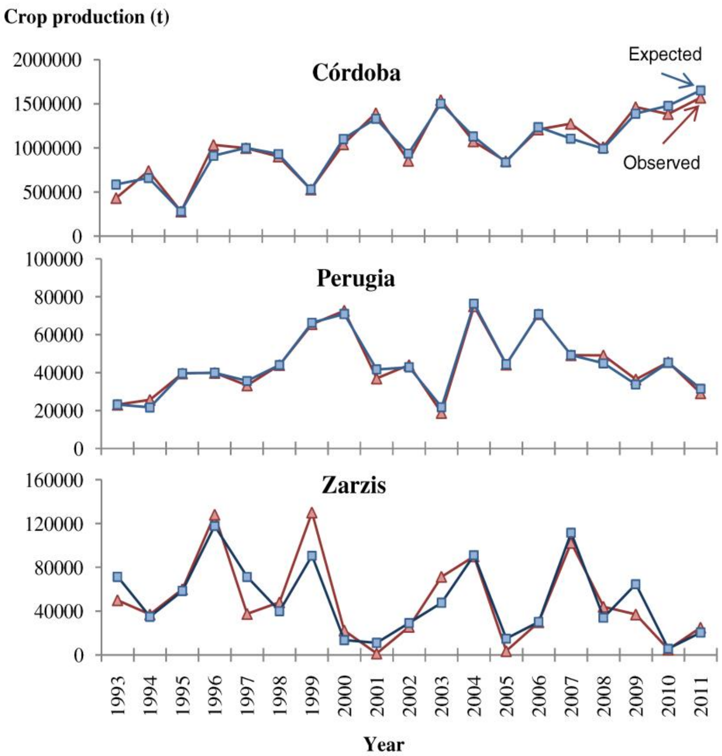

Fig. 4 Yearly crop production as observed and as expected according to the indicated local models; *t* metric tons. These models were validated using the full cross-validation. As can be seen, these local models can be used effectively for crop forecasting purposes

The Intergovernmental Panel on Climate Change (IPCC WGII 2007) proposes several future climatic scenarios based on different estimations of humanity development and climate trends. The A1B emission scenario is the most accepted by the scientific community; this future scenario assumes a rapid increase of greenhouse gas (having about 700 ppm of CO2 concentration in year 2010). Giorgi and Lionello (2008) use this scenario for concluding that temperatures and evapotranspiration increases in the Mediterranean basin opposite to rainfall that decreases. In this sense, according to present results, olive crop production will tend to decrease although local climatic features can modify the way in which olive trees will respond to climate change. For this reason, the present paper must be taken into account in future studies to confirm this fact.

The data of this study are important for determining actual meteorological requirements of different olive cultivars in the Mediterranean basin where olive cultivation is the main resource for thousands of families and generate great economic wealth each year. Due to the novelty of the statistical approach on crop modelling and the big surface extension analysed in the present paper, the study has improved the results obtained in the present, being of vital importance from the standpoint of environmental, economic and social development in a changing environment.

4 Conclusions

The local models had potentially high predictive values, while these predictive values appeared to decrease in national models along with increases in the heterogeneity of the characteristics of the overall study area. In this sense, we propose the use of specific low-scale models better adapted to agroclimatic features with forecasting purposes.

However, national models provide valuable information about the parameters that had greater effects on olive crop forecast in the different study areas and about the areas that will be more fragile in a changing environment.

The pollen index and water availability during spring represented the most significant variables in all models, and they were essential for optimal crop production in the Mediterranean basin. Temperature has been another significant parameter in the models with clear positive effects on crop production during the period prior to flowering and during autumn, and with negative effects on crop production during the process of fruit setting in winter and then during summer.

This study has improved all the previous crop modelling studies under aerobiological approach in a statistical sense and on the spatial extrapolation of conclusions, providing a partial least square regression analysis applied to a broader Mediterranean surface, i.e. the largest olive growing area in the world. The present paper proposes a new approach that improves all previous studies. This new statistical approach shows better results than with previous olive crop models based on linear regression; it should be taken into account for future crop modelling studies in different agroecosystems.

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8. CONCLUSIONES

Discusión general

Los resultados del presente trabajo, enfocado en el estudio del ciclo reproductor del olivo, explican en gran medida muchos de los factores que influyen en los principales eventos fenológicos de éste. Así, los capítulos I y II se enfocan en el análisis de la intensidad de la floración, desarrollando modelos predictivos para la provincia de Córdoba en base a 30 años de datos. En éstos se caracteriza estadísticamente la relación de la intensidad polínica, expresada como Índice Polínico, con las condiciones ambientales logrando una antelación en su predicción de hasta 2 meses. Éste tipo de modelos son conocidos como modelos de predicción a largo plazo. Aunque el Índice Polínico es uno de los parámetros aerobiológicos más importantes, son escasos los estudios previos que lo modelizan e intentan predecirlo. Estos trabajos usualmente utilizan un análisis de regresión lineal múltiple para explicar su relación con las condiciones ambientales, como por ejemplo en el caso de *Betula* (Dahl y Strandhede 1996; Puc, 2012), *Quercus* (Corden y Millington, 1999) y *Poaceae* (Emberlin et al., 1999; Smith y Emberlin, 2006; García-Mozo et al., 2010b; Sabariego et al., 2012).

En el caso del olivo, también la mayoría de trabajos que intentan modelizar su Índice Polínico, usan el análisis de regresión lineal múltiple. A través de esa metodología (Recio et al., 1996; Fornaciari et al., 1997; Galán et al., 2001b; Ribeiro et al., 2006b) indican cómo la precipitación y la temperatura registrada durante los meses previos al inicio de la floración son los parámetros meteorológicos que más influyen sobre el Índice Polínico del olivo. Estos resultados coinciden en gran parte con los resultados obtenidos en la presente tesis, aunque ésta emplea un enfoque estadístico diferente a los anteriores que permite extraer información más precisa de los datos. Se ha aplicado una estadística integradora basada en: el uso de índices biometeorológicos y autorregresivos, capaces de representar mejor los efectos biológicos de las distintas variables ambientales; en un sistema de optimización de reglas que tiene en cuenta los efectos de eventos meteorológicos extremos; y finalmente, en un “three step method” que agrupa los casos de estudio de acuerdo a sus similitudes biometeorológicas para poder construir modelos más ajustados. Esta metodología integrada ha permitido obtener otras nuevas conclusiones. Aunque temperaturas moderadas durante marzo ya se habían descrito como relacionadas con una mayor intensidad de floración (Ribeiro et al. 2006), se ha observado que temperaturas excesivamente elevadas pueden producir un estrés térmico en la planta. También cómo temperaturas extremadamente bajas durante el periodo de dormancia pueden ser perjudiciales para la floración, lo que había sido anteriormente descrito por otros autores (Bartolozzi y Fontanazza, 1999; Orlandi et al., 2004). En la presente tesis también se observa una correlación positiva entre el número de días de lluvia y la intensidad de floración, ya que el modo en que se produce la lluvia puede afectar a la disponibilidad hídrica (Ribeiro et al., 2006b). Además se pone de manifiesto cómo las condiciones acontecidas durante los meses estivales poseen gran influencia sobre la intensidad de la floración del año siguiente. Otros autores han observado que temperaturas anormalmente elevadas durante verano benefician la producción de polen en primavera (Mandrioli, 1987). Éste hecho puede ser atribuible al solapamiento fenológico que sucede entre el crecimiento vegetativo y reproductor en el caso del olivo y al carácter inhibidor de la floración que posee el fruto recién formado, de este modo unas malas condiciones durante el periodo de fructificación podrían disminuir la competencia para el crecimiento vegetativo y permitir una mayor número de yemas inducidas para la floración (Rallo y Cuevas, 2004; Dag et al., 2010).

No se debe olvidar, como han indicado diferentes autores (i.e. Fornaciari et al., 1997), que las condiciones meteorológicas que se registran durante la estación polínica poseen un considerable efecto sobre el Índice Polínico. Sin embargo, estas condiciones no pueden incluirse en la creación de modelos de pronóstico ya que desaparecería el carácter predictivo de los mismos.

En el capítulo I se producen tres importantes avances en cuanto a la modelización a largo plazo. En primer lugar se ha diseñado una metodología para la utilización de índices biometeorológicos con la finalidad de expresar las variables ambientales de un modo integrado que represente mejor sus efectos sobre los sistemas biológicos. Gracias al uso combinado de factores ambientales en forma de índices se puede obtener un valor predictivo mayor que el ofrecido por el uso aislado de éstos (Schwartz, 1990; Schwartz y Reiter, 2000; Valencia-Barreda et al., 2002).

En segundo lugar se han creado un sistema de optimización de los índices biometeorológicos basado en reglas mediante “bonificaciones” y “penalizaciones”.

Esto ha permitido entender mejor los efectos de los eventos meteorológicos extremos sobre la intensidad de la floración. Ésta optimización se ajusta dentro de los Sistemas de Reglas (Setnes et al., 1998), los cuales se basan primero en una formulación de condicionantes lógicos, segundo realizan una comparación de resultados bajo estos supuestos y tercero, se aplican bonificaciones o penalizaciones en función de los resultados. Estos sistemas han sido muy utilizados en otras áreas del conocimiento, como la Econometría (i.e. Gordy, 2003). Tradicionalmente se ha enfocado la relación entre el ambiente y sus efectos sobre los sistemas biológicos mediante una metodología estadística lineal, pero esta relación sólo es puramente lineal en los casos donde los valores en las variables ambientales se sitúan en el rango medio de lo registrado habitualmente durante la historia evolutiva de la especie. Cuando se producen efectos meteorológicos extremos la relación estadística lineal se rompe y no representa la relación real entre la meteorología y la respuesta biológica. Gracias a la optimización de los índices se ha conseguido modelizar el efecto de eventos meteorológicos extremos que caracterizan al clima mediterráneo, conociendo el impacto de temperaturas anormalmente frías o de la lluvia torrencial sobre el comportamiento reproductor del olivo.

En tercer lugar se desarrolla un Índice de Ciclicidad que contempla el patrón recurrente en la producción de polen de olivo a través de los años y la naturaleza autorregresiva de la serie temporal. Esta característica es producto de la dependencia que posee la intensidad de la floración de un años con la de los años anteriores dadas las características del ciclo reproductor del olivo (Ramos Dos Santos, 2000). Además, el comportamiento cíclico ha sido atribuido a varios otros motivos, por un lado se encuentran todas las causas responsables del comportamiento vecero del olivo (Lavee, 2007), y por otro lado también puede ser un reflejo del patrón climático del clima Mediterráneo (Lionello et al., 2006).

La construcción de modelos autorregresivos había sido enfocada anteriormente para realizar predicciones a corto plazo, pero no existían antecedentes en predicciones a largo plazo, posiblemente debido a la necesidad de series temporales largas (Arca et al., 2002; Belmonte y Canela, 2002; Rodríguez-Rajo et al., 2006).

En el capítulo II se ha desarrollado un método denominado “three step method” con la finalidad de considerar las diferencias que poseen los mismos parámetros meteorológicos en cuanto a la influencia sobre los mismos individuos, dependiendo de las características meteorológicas de un periodo anterior, ya que éstas condiciones anteriores pueden incidir sobre la sensibilidad del olivo ante el ambiente. De este modo se pretende construir modelos mejor ajustados a unas condiciones ambientales determinadas. El método consiste en realizar primero una agrupación de los años en base a las características de la floración y de la meteorología anual, en segundo lugar desarrollar un modelo de clasificación en base a la meteorología previa a la estación polínica y en tercer lugar aplicar un modelo de predicción específico en cada categoría.

La agrupación se ha llevado a cabo mediante los métodos conglomeración jerárquica y conglomeración K-medias. Los métodos de conglomeración son muy empleados en ciencias naturales, como la Ecología o la Aerobiología (Sánchez-Mesa et al., 2002, 2005). Durante el segundo paso, han sido testados diferentes métodos de clasificación, siendo las redes neuronales artificiales el método seleccionado. Otros autores han comparado también diversos métodos de clasificación, como el análisis discriminante o los árboles de decisión, obteniendo resultados similares a los de la tesis (Aznarte et al., 2007; Kaspryk et al., 2011; Voukantsis et al., 2010; Rodríguez-Rajo et al., 2010; Sánchez-Mesa et al., 2005; Puc, 2012). La modelización ha sido desarrollada mediante regresión por el método de los mínimos cuadrados parciales (PLSR), un método utilizado por unos pocos autores en estudios fenológicos, pero nunca en estudios aerobiológicos (Reynolds et al., 2002; Yu et al., 2010).

Los resultados obtenidos en los capítulos I y II han supuesto un importante avance en el campo de la “modelización a largo plazo”, ofreciendo conocimientos adicionales muy destacados sobre la floración del olivo.

El capítulo III se enfoca más al estudio de la fenología directa de campo, y así en primer lugar muestra una comparación estadística de la efectividad del muestreo fenológico en diferentes niveles de temporalidad. La periodicidad óptima de los muestreos fenológicos ha sido pocas veces analizada, y aunque ésta pueda depender de varios factores tales como el tipo de especie analizada, el periodo fenológico muestreado o el área geográfica es un factor de vital importancia en la planificación de estudios de fenología (Schwartz, 1994, 2003).En bibliografía encontramos gran variedad de métología temporal. Algunos trabajos utilizanobservaciones fenológicas anuales (Peñuelas et al., 2002), otros mensuales (Palacio y Montserrat-Martí, 2005), bisemanales (Patel, 1997), semanales (León-Ruíz et al., 2012) o incluso diarias, cuya dificultad de monitoreo se ha visto recientemente reducida a causa del empleo de nuevas técnicas como el análisis de imágenes NVDI (Zhang et al., 2003).

Trabajos previos en fenología reproductora de olivo se basan normalmente en muestreos semanales (Vázquez, 2000; García-Mozo et al., 2006; Aguilera y Ruiz-Valenzuela., 2009; Rojo y Pérez-Badía, 2013). Nuestros resultados revelan que, un seguimiento semanal no tiene diferencias significativas con respecto a un seguimiento diario. Sin embargo, un intervalo de tiempo superior proporcionaría datos significativamente diferentes a los reales.

Otro objetivo perseguido en este capítulo se ha basado en la búsqueda de las causas externas más importantes en la configuración de las características fenológicas propias de una determinada zona. A nivel macroclimático se considera a la latitud como el principal factor responsable de la caracterización fenológica de un área determinada (Orlandi et al., 2010d), sin embargo, a nivel microclimático la latitud deja de jugar un importante papel, identificándose a la altitud como la principal responsable (Fornaciari et al., 2000; García-Mozo et al., 2006; Aguilera y Ruiz-Valenzuela, 2009; Rojo y Pérez-Badía, 2013). En el presente trabajo se corrobora que la altitud es el factor topográfico más importante, identificando a la orientación Este-Oeste de la ladera como otro factor determinante del desarrollo fenológico.

Se ha analizado también la relación entre la evolución fenológica interanual y distintas variables meteorológicas, tratando de identificar los parámetros que más pueden influir sobre el desarrollo fenológico. Se ha presentado a la temperatura como la variable meteorológica más importante para el inicio del desarrollo fenológico del olivo, corroborando los resultados de otros autores (Galán et al., 2005; Orlandi et al., 2012). El déficit hídrico posee claros efectos sobre el desarrollo del ciclo reproductor del olivo, en el presente trabajo se ha demostrado además que una baja disponibilidad hídrica para la planta en el inicio del desarrollo fenológico puede producir un retraso generalizado en todas las fenofases hasta el final de la floración (Rapoport et al., 2012; Moorthy et al., 2011).

Por último, en el capítulo IV se ha modelizado la producción de fruto en una amplia área de la Cuenca del Mediterráneo. Otros autores ya han realizado predicciones de cosecha en plantas anemófilas utilizando el Índice Polínico y las condiciones meteorológicas previas como variables predictoras, identificándose siempre el polen aerovagante como la variable más importante para obtener una modelización óptima (i.e. Candau et al., 1998; González-Minero et al., 1998; Fornaciari et al., 2002; Galán et al., 2004). Aunque los trabajos previos comparten el empleo de regresión lineal para tratar de predecir la producción de cosecha, existen ciertos aspectos que los diferencian. Así en Italia aparece el empleo del periodo de polinización efectivo y de parámetros bioclimáticos (Fornaciari et al., 2005; Orlandi et al., 2005, 2010), el uso de variables fenológicas y del aporte vecero en España (Galán et al., 2008, García-Mozo et al., 2008, Aguilera, 2012) o el empleo de variables fenológicas y de modelos con varias zonas de muestreo polínico en Portugal (Ribeiro et al., 2007, 2008).

El presente trabajo se diferencia de los antecedentes, primero, en el empleo de un nuevo método estadístico de modelización de de cosecha, la regresión por el método de los mínimos cuadrados parciales. Este método es más apropiado cuando existe un gran número de variables predictoras y es capaz de extraer más información (Carrascal et al., 2009). En segundo lugar, también se ha modelizado a gran escala la producción de fruto en la Cuenca del Mediterráneo con la finalidad de obtener conclusiones más globales.. Así, durante el estudio se han desarrollado modelos ajustados a dos escalas diferentes: (i) modelos locales que poseen un gran valor predictivo ya que están bien ajustados a las condiciones microclimáticas del lugar; (ii) modelos regionales que aportan una valiosa información acerca de los requerimientos bioclimáticos del olivo en el área mediterránea. Los modelos locales ofrecen un poder predictivo mucho mayor pero sólo son aplicables a pequeña escala, sin embargo los modelos regionales ofrecen información mucho más general cuya aplicabilidad depende de su área de aplicación y de su ajuste y robustez (Chuine et al., 2000).

Coincidiendo con los antecedentes estudios, se identifica el Índice Polínico como la variable con más peso en los modelos de predicción (i.e, Galán et al. 2004, 2008). Además se observa la importancia de la disponibilidad hídrica en primavera para la formación de fruto (precipitación acumulada menos la evapotranspiración entre octubre y abril) (Rallo, 1994; Candau et al., 1998; Galán et al., 2004, 2008). El déficit hídrico puede provocar graves daños en diferentes estructuras del olivo (Bacelar et al. 2007, 2009; Barranco et al. 2008; Rapaport et al. 2012). También se observa la importancia de que se produzcan unas condiciones térmicas apropiadas durante el comienzo del verano (coincidiendo con el proceso de cuajado de fruto) y durante todo el otoño (coincidiendo con el proceso de maduración de fruto) (García-Mozo et al., 2008; Oteros et al., 2013b; Aguilera et al., 2013). El registro de temperaturas extremas durante estos periodos muestra una correlación negativa con la producción de cosecha como ha sido observado con anterioridad (Fornaciari et al., 2005; Orlandi et al., 2006).

Los estudios que se han llevado a cabo en base a los objetivos propuestos ponen de manifiesto que el olivo posee un patrón fenológico muy adaptado al clima mediterráneo con un crecimiento vegetativo y reproductor concentrado en primavera y otoño, coincidiendo con condiciones meteorológicas menos extremas. Su ciclo reproductor entra en dormancia para resistir las adversidades del clima y requiere un estímulo de frio durante el invierno para salir de la misma. La intensidad de la floración y el desarrollo fenológico se ven favorecidos por precipitaciones moderadas y temperaturas suaves en primavera. La producción de fruto es más elevada cuando las condiciones meteorológicas registradas durante el año se encuentran dentro de lo habitual y no se producen alteraciones extremas. Se concluye que el olivo es una especie eminentemente mediterránea que posee una gran resiliencia; pero su productividad puede verse afectada por cambios en el clima.

Conclusiones

Capítulo I

Biometeorological and autoregressive indices for predicting olive pollen intensity.

1. El modelo de índices propuesto posibilita la detección y cuantificación de la influencia que ejercen los eventos meteorológicos extremos sobre la floración de olivo. La aplicación de este tipo de análisis podría suponer un importante avance en la modelización de la fenología de especies árboreas, y en especial de las mediterráneas, sometidas a las fuertes variaciones del clima.

2. El índice de Ciclicidad supone un avance en la modelización aerobiológica a largo plazo, ya que tiene en cuenta el comportamiento autorregresivo de la serie temporal del Índice Polínico.

3. Los índices explicativos con más peso en el modelo de floración incluyeron variables como la temperatura durante invierno y primavera temprana, la precipitación acumulada antes de la estación polínica, la temperatura durante el verano y la tendencia autorregresiva de la floración.

Capítulo II

Year clustering analysis for modelling olive flowering phenology.

4. Se ha comprobado la efectividad del “Three step method” en la modelización aerobiológica a largo plazo. Su efectividad radica en que los años agrupados en una determinada categoría comparten características fenológicas similares ya que la especie objeto de estudio han sido sometidas a condiciones ambientales similares en el área de estudio.

5. Tanto el análisis de regresión por el método de los mínimos cuadrados parciales cómo las redes neuronales artificiales se revelaron como métodos apropiados para desarrollar modelos fenológicos de clasificación apropiados.

6. Se corrobora el importante efecto de la temperatura durante el invierno y la primavera temprana, el de la disponibilidad hídrica, así como el de la temperatura estival sobre la intensidad de la floración del olivo.

Capítulo III

Modelling olive phenological response to weather and topography.

7. El análisis estadístico para determinar la periodicidad óptima en el seguimiento de la fenología floral del olivo muestra que un muestreo semanal resulta válido y aceptable.

8. Las variables topográficas que más influyen sobre la fenología reproductora son la altitud y la orientación Este-Oeste de la máxima pendiente de la ladera. Los parámetros meteorológicos más importantes para el desarrollo fenológico son la temperatura durante invierno y primavera temprana así como la disponibilidad hídrica durante el proceso de desarrollo de los órganos reproductores.

9. Un conocimiento detallado acerca de la influencia de las condiciones bioclimáticas sobre el desarrollo fenológico del olivo es una información muy útil para comprender los efectos que podrá tener el cambio climático global sobre el olivo y para estimar las posibilidades de adaptación del olivo a otras áreas geográficas.

Capítulo IV

Better prediction of Mediterranean olive production using pollen-based models.

10. Los modelos de predicción adaptados a condiciones locales poseen un elevado valor predictivo, sin embargo, este carácter predictivo desciende a medida que se incrementa la heterogeneidad del área modelizada.

11. Los modelos globales aportan una información muy útil para entender los efectos del ambiente sobre el olivo en las diferentes áreas geográficas de la Cuenca Mediterránea, así como identificar las áreas más vulnerables ante un posible cambio del clima.

12. Las variables con mayor peso en los modelos de predicción de cosecha han sido el Índice Polínico y la disponibilidad hídrica en primavera. La temperatura ha sido otro parámetro significativo en gran parte del Mediterráneo, habiéndose observado una correlación positiva entre la temperatura registrada durante el periodo previo a la floración y durante el otoño, y una correlación negativa durante el periodo de cuajado de fruto y durante el verano.

Conclusions

Chapter I

Biometeorological and autoregressive indices for predicting olive pollen intensity .

1. The index-based model used here enabled the influence of extreme weather events on olive flowering to be detected and quantified. Application of this kind of analysis may represent a major advance in the phenological modelling of tree species, particularly in the Mediterranean area, which is subject to marked changes in weather patterns.

2. The Cyclicity Index represents a step forward in long-term aerobiological modelling, since it takes into account the autoregressive behaviour of the Pollen Index time series.

3. The indices most influencing the flowering model included a number of weather-related variables: winter temperature, early spring temperature, accumulated rainfall prior to the pollen season, summer temperature and the autoregressive trend shown by flowering.

Chapter II

Year clustering analysis for modelling olive flowering phenology.

4. The “three-step method” proved effective for long-term aerobiological modelling, since years grouped in a given category shared similar phenological characteristics, given that the study species is subject to similar environmental conditions throughout the study area.

5. Both partial least squares regression and artificial neural networks proved suitable for the construction of appropriate phenological classification models.

6. Findings confirmed the crucial effect of temperatures in winter and early spring, water availability and summer temperatures on olive flowering intensity.

Chapter III

Modelling olive phenological response to weather and topography.

7. Statistical analysis performed to determine the optimal timing for the monitoring of olive floral phenology showed that weekly sampling provided reliable and acceptable results.

8. The topographical variables most influencing olive reductive phenology were altitude and the East-West orientation of the steepest slope. The most important weather-related factors for phenological development were temperatures in winter and early spring, and water availability during reproductive organ development.

9. Detailed knowledge of the influence of bioclimatic conditions on olive phenological development is of great value for understanding the potential effects of global climate change on the olive, and for estimating the scope for the olive's adaptation to other geographical areas.

Chapter IV

Better prediction of Mediterranean olive production using pollen-based models.

10. While prediction model adapted to local conditions display high predictive value, this value diminishes as the heterogeneity of the area modelled increases.

11. Global models provide valuable information for understanding the effects of the environment on the olive in different areas of the Mediterranean Basin, and for identifying the areas most vulnerable to potential climate change.

12. The variables most influencing harvest prediction models were the Pollen Index and water availability in spring. Temperature also exerted a considerable effect on harvest size over most of the Mediterranean area, a positive correlation being observed between harvest size and temperatures in the period prior to flowering and during the autumn, and a negative correlation between harvest size and temperatures both during fruit set and in the summer.

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10. Anexos

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Statistical approach to the analysis of olive long-term pollen season trends in southern Spain

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HIGHLIGHTS

- A long term olive airborne pollen analysis has been performed.
- Three types of statistical analysis were performed and compared: Linear Regression, Seasonal-Trend Decomposition procedure based on Loess (STL), and an ARIMA model.
- Olive Pollen Index is significantly increasing in South Europe.
- There is a lengthening of the Pollen season mostly due to the advance of the Pollen Season Start.
- The combination of the lengthening of the season with the increase in airborne pollen counts can play a negative effect on pollen-allergy sufferers in the Mediterranean area.

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ABSTRACT

Analysis of long-term airborne pollen counts makes it possible not only to chart pollen-season trends but also to track changing patterns in flowering phenology. Changes in higher plant response over a long interval are considered among the most valuable bioindicators of climate change impact. Phenological-trend models can also provide information regarding crop production and pollen–allergen emission. The interest of this information makes essential the election of the statistical analysis for time series study.
We analysed trends and variations in the olive flowering season over a 30-year period (1982–2011) in southern Europe (Córdoba, Spain), focussing on: annual Pollen Index (PI); Pollen Season Start (PSS), Peak Date (PD), Pollen Season End (PSE) and Pollen Season Duration (PSD). Apart from the traditional Linear Regression analysis, a Seasonal-Trend Decomposition procedure based on Loess (STL) and an ARIMA model were performed.
Linear regression results indicated a trend toward delayed PSE and earlier PSS and PD, probably influenced by the rise in temperature. These changes are provoking longer flowering periods in the study area.
The use of the STL technique provided a clearer picture of phenological behaviour. Data decomposition on pollination dynamics enabled the trend toward an alternate bearing cycle to be distinguished from the influence of other stochastic fluctuations. Results pointed to show a rising trend in pollen production.
With a view toward forecasting future phenological trends, ARIMA models were constructed to predict PSD, PSS and PI until 2016. Projections displayed a better goodness of fit than those derived from linear regression.
Findings suggest that olive reproductive cycle is changing considerably over the last 30 years due to climate change. Further conclusions are that STL improves the effectiveness of traditional linear regression in trend analysis, and ARIMA models can provide reliable trend projections for future years taking into account the internal fluctuations in time series.

1. Introduction

Reproductive phenology is now recognized as a major indicator of the impact of climate change (Menzel and Sparks, 2006). In the northern hemisphere, the growing season is becoming longer; spring is starting earlier, and autumn later (Ahas et al., 2002; Menzel, 2000). This trend is well documented in northern Europe and North America, but less is known about Mediterranean species (Peñuelas et al., 2002).

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Application of appropriate statistical techniques to long-term phenological data enables fluctuation patterns and overall trends to be analysed. One of the most widely-used techniques has traditionally been linear regression (Bradley et al., 1999; García-Mozo et al., 2010). Nevertheless, phenological data do not always fit a linear regression model, since frequent year-on-year variations give rise to non-linear patterns.

The present study analyzes olive flowering phenology on the basis of airborne pollen data for southern Spain over a recent 30-year period (1982–2011). The olive tree is a species well adapted to wind pollination which releases large amounts of pollen into the atmosphere. Airborne pollen from anemophilous species is an important phenological data. This data can be used not only for crop management purposes in agronomic or forestry species (García-Mozo, 2011), but also for the adoption of preventive measures in the case of allergenic pollen grains as is the case of olive species (Galán et al., 2013; Oteros et al., 2013a). Moreover these phenological data are key indicators of the impact of climate change (Bonofiglio et al., 2013).

The region of Andalusia (southern Spain) boasts the world's largest area of olive-groves that produces over 5 million tonnes of olive fruits per year. In this region, the province of Córdoba accounts for a third of all Andalusian cropland being the second largest producer province. The olive monoculture expansion and the high number of allergens present in olive pollen grains have given rise to atopy and asthma in a large proportion of the population throughout the Mediterranean area (Barber et al., 2008).

Research has confirmed that in wind-pollinated species, both pollination timing and also intensity, expressed by the Pollen Index (PI) as the annual sum of daily airborne pollen counts, vary considerably from year to year due not only to weather-related factors but also to a number of species-specific characteristics (Jäger, 1989). In the case of PI, regular alternate bearing cycles have been reported for several wind-pollinated tree species in northern Europe, including *Betula* and *Alnus* (Andersen, 1980; Nilsson and Persson, 1981) and *Quercus robur* (Emberlin et al., 1993). In the Mediterranean area, this cycle is much less common, due to the extreme year-on-year variations in temperature and rainfall typical of the Mediterranean climate. Alternate bearing cycle is well described in olive tree (Lavee, 2007) and research on *Olea europaea* pollination suggests the same alternate cycle (Domínguez et al., 1993; Galán et al., 2004; Barranco et al., 2008), although it can be interrupted due to extreme events like water-stress years (Díaz de la Guardia et al., 2003; Galán et al., 2008).

Analysis of fossil-pollen records has shown that such trends can indicate ecological or anthropogenic changes, albeit over periods of hundreds of years (Grimm, 1988). To date, few studies have focussed on long-term airborne pollen trends (Recio et al., 2009; Emberlin et al., 2002; Ziello et al., 2012). Continuous pollen monitoring started recently in most places; nevertheless, some papers so far reveal changes not only in annual pollen intensity but also in the timing of pollination, due to increasing temperatures and variations in rainfall patterns (Menzel, 2000; Ziello et al., 2012). In the case of *Olea* pollen, some studies have detected a general advance of the pollen season starting in the Mediterranean area (Aguilera et al., 2013; Avolio et al., 2012; Bonofiglio et al., 2009; Sicard et al., 2012; Orlandi et al., 2013a, 2013b), including different sites in South Spain where pollen detection started in 1982 in Córdoba city and from 1992 in other provinces (Díaz de la Guardia et al., 2003; Galán et al., 2005, García-Mozo et al., 1999).

The statistical techniques usually employed to study pollen-season trends are correlation and regression analyses, even though the interpretation of results is governed by the normality and linearity of the data used. The present study sought to explore other methods for analysing trends in olive pollen data, including a Seasonal-Trend Decomposition procedure based on Loess (STL) taking into account the olive's alternate reproductive pattern and ARIMA models which produce a forecasting model on the basis of autoregressive analysis. The main characteristics of the pollen season were analysed including start, peak, and end dates, duration of pollen season and the variations on annual intensity expressed as PI. Improving forecasting of the olive flowering intensity in southern Europe will be of great value for both crop-management and allergy-prevention purposes.

Long-term trends in olive flowering phenology were analysed using a 30-year database of *Olea* pollen records for Córdoba. The main aim of the study is to detect the potential trends on olive reproductive phenology in the last years in South Europe. For this purpose we have compared different statistic methods in order to offer the most reliable results: 1. to chart long-term trends in olive flowering phenology using linear regression; 2. to analyse long-term trends in pollen season using a Seasonal-Trend Decomposition procedure based on Loess; and 3. to forecast future trends in the annual pollen index, the pollen-season start date and the pollen-season duration using ARIMA models.

2. Material & methods

The study used a 30-year database (1982–2011) of pollen records for the city of Córdoba (southern Spain). The area has a Mediterranean climate with some continental features. The annual mean temperature is 17.8 °C and the annual average rainfall is 621 mm.

Phenological data on flowering intensity were measured by analysing airborne olive pollen counts, using a Hirst-type volumetric spore trap (Hirst, 1952) placed on the roof of the Educational Sciences Faculty, at 15 m above ground level. This sampler captures olive pollen over a radius of 100 km, thus providing a good indication of flowering phenology for the whole province of Córdoba (Hernández-Ceballos et al., 2010). Pollen counts were obtained using a standard protocol published by the Spanish Aerobiology Network (REA) (Galán et al., 2007). Data on the following phenological features were extracted for each study year: Pollen Season Start (PSS), Peak Date (PD), Pollen Season End (PSE), and Duration (PSD), as well as the annual Pollen Index (PI). The PSS was defined as the first day on which we record ≥ 1 pollen grain/m^3 and also in the following 5 days ≥ 1 pollen grain/m^3 per day are recorded (García-Mozo et al., 1999). The PSE was the last day on which 1 pollen grain/m^3 was recorded and counts on subsequent days were 1 or 0 pollen grain/m^3. The PI is defined as the sum of the daily average pollen counts per cubic metre throughout the year. The PD was defined as the day on which the highest daily pollen count was recorded.

Analysis of long-term trends in the olive reproductive cycle was performed using three approaches. Apart from linear regression, a Seasonal-Trend Decomposition procedure based on Loess (STL) technique was performed, taking into account olive reproductive patterns. An ARIMA model was also constructed for modelling and forecasting some pollen season features. ARIMA models enable long-term forecasting on the basis of autoregressive analysis, in which predicted values for the output variable are based on its own previous values, regardless of external factors such as potential weather patterns:

1 Linear regression analysis was used to study long-term trends displayed by the following phenological features: PSS, PD, PSE, PSD and PI.
2 A Seasonal-Trend Decomposition Procedure based on Loess (STL) was performed. STL is a filtering procedure for decomposing a time-series into additive components of variation (trend, seasonality and irregularity) by the application of loess smoothing models (Cleveland et al., 1990; Chaloupka and Limpus, 2001). This technique was applied to the daily-pollen time series with a view to analysing trends in daily pollen production over time, since some components of pollen time series produce distortions impeding our understanding of their long-term behaviour.

The daily-pollen time series was regarded as a mixture of several components: Trend (T), Seasonal (S) and Irregular (I). A decomposition technique was used to distinguish these components in study data, and to analyse only the Trend component, thus eliminating distortions in the interpretation of results, P being the daily-pollen time series:

$$P = T + S + I.$$

3 In order to forecast PI, PSS and PSD until 2016, ARIMA (Autoregressive Integrated Model of Running Mean) univariate seasonal and non-seasonal models (also known as "Box–Jenkins" models) were applied. Three parameters were tested: Autoregressive (p), Differentiation (d) and Running mean (q):

p Number of autoregressive terms. Each term measures the independent effect of the values with a specified lag. An order 2 autoregressive means that each value of the series is affected by the two preceding values (regardless of each other).

d Number of times that a time series was transformed calculating the differences between the values of the series and its predecessors.

q The order of the running mean of the process.

3. Results

3.1. Study of phenological and meteorological trends using linear regression models

Table 1 shows the results of the trend analysis performed to the climatic data of Córdoba city recorded during the studied period, 1982–2011. Slopes of the linear regression trend analysis of temperatures and rainfall show an increase of both parameters, overall in the case of spring temperature. Data for the PSS, PD and PSE of the pollen season are shown in Fig. 1. Both PSS and PD appear to have occurred earlier over recent years, while PSE has been occurring later; the delay in PSE is 0.8 day per year ($p = 0.01$). As the graphic shows, the result is that pollen season is lasting longer, largely due to delayed PSE. The advance in PSS is less significant than the trend shown by PSE. The combination of the two trends means that the pollen season has been lengthened by over one day per year. The PD for any given year is also expected to occur 0.75 day earlier than in the previous year ($p = 0.0017$).

Regression analysis (Table 2) indicated a significant increase in annual pollen count; the PI has been increasing on average by 699 per year. So, while the season may start slightly earlier, the peak is reached sooner, even though the season is lasting longer, and annual pollen output is rising lengthening in time and the annual pollen is increasing: i.e. there is more pollen over more days.

Table 1
Slopes of the linear regression trend analysis of Maximum (TMx), Minimum (TMin) and Mean Temperature (TMn) in °C, and rainfall (Rf) during the studied period (1982–2011) in the city of Córdoba.

Meteorological parameters	January–March	April–June	January–June	January–December
TMx	0.014	0.084*,**	0.050**	0.033**
TMin	0.007	0.071***	0.057**	0.043**
TMn	0.011	0.077***	0.054***	0.038***
Rf	8.139	0.516	2.982	4.176

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

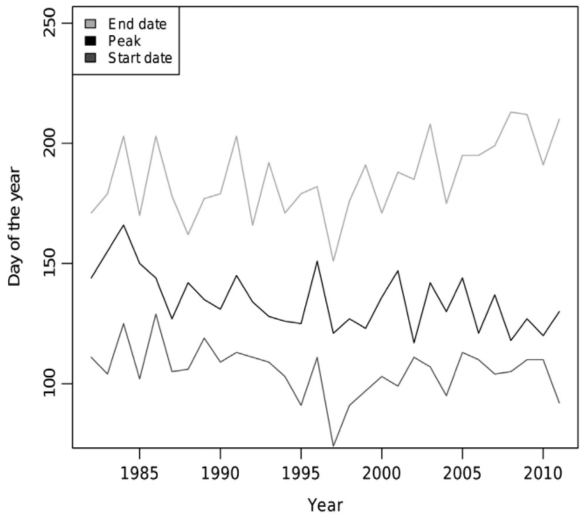

Fig. 1. Variation of Pollen Season Start, Peak Date, and Pollen Season End dates along the study period (1982–2011).

3.2. Seasonal-Trend Decomposition procedure based on Loess to study trends in pollen time series

The seasonal decomposition by loess, a method of regression with a weighted polynomial that is localized for different parts of the data, is shown in Fig. 2. By removing the seasonal trend, what is left is a much more obvious upward trend over time. The seasonal component shows variations in daily-pollen time series as a result of annual climate patterns. The reproductive cycles of organisms are usually adapted to the annual seasonality of the weather, and maintain annual patterns. The remainder component also shows variations due to random weather-related features which, in the Mediterranean region, display considerable year-on-year variability. Finally, the trend component charts the real long-term trend in atmospheric pollen content over time, clearly displaying a rising slope.

Fig. 3 shows trend with seasonality and randomness removed, together with a linear analysis of the trend component of the daily-pollen time series. This analysis confirms the positive trend in annual pollen production. Regression analysis shows that PI is increasing significantly ($p < 0.001$).

3.3. ARIMA models based on Box–Jenkins procedure

Linear regression and ARIMA analyses were applied to PI, PSS and PSD, as the most important variables on olive reproductive phenology. Based on the values of the auto-covariance function (ACVF) and the autocorrelation function (ACF) a Box–Jenkins model was constructed (Table 3). Log likelihood (Log lik.), the Akaike Information Criterium (AIC) and the Bayesian information criterion (BIC) were the adjustment criteria used for selecting the best model in each case. ARIMA analyses (Fig. 4) provided a more accurate model for PI and PSS. The AR1 parameter indicated that PI model was based on the existence of autocorrelation between the PI of a given year (t) and that of the previous year

Table 2
Linear regression summary: PSS, Pollen Season Start; PD, Peak Date; PSE, Pollen Season End; PSD, Pollen Season Duration; PI, Pollen Index.

	PSS	PD	PSE	PSD	PI
R^2	0.09	0.30	0.21	0.47	0.42
Slope	−0.36	−0.75	0.83	1.19	699
p	0.1071	0.0017	0.0112	0.0000	0.0001

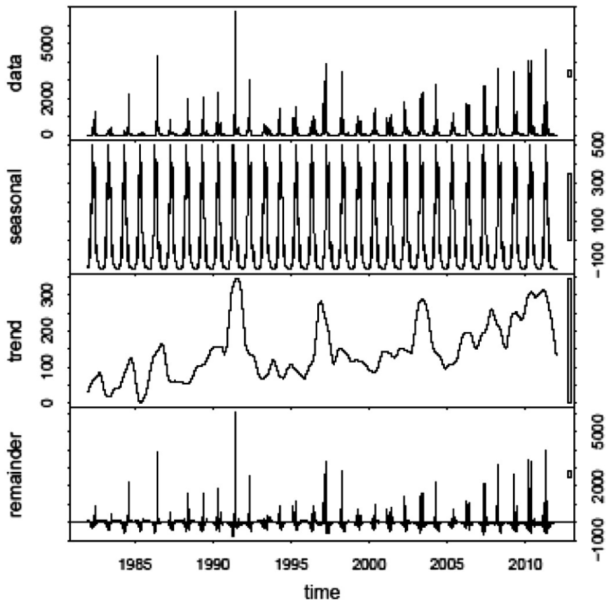

Fig. 2. Seasonal-Trend Decomposition procedure based on Loess. Daily pollen time series, seasonal component of time series, trend component of time series and remainder component of time series.

(t − 1). Similarly, the PSS model was based on the existence of autocorrelation between PSS_t and PSS_{t-1}. But the two models also had a SAR1 parameter, meaning that both variables display clearly seasonal behaviour. The PSD model also had AR1 and SAR1 parameters, but additionally presented an AR2 parameter meaning that the model was based on the existence of autocorrelation between PSD of a given year (t) and that of the year t − 2. Forecasting of future PSD by ARIMA (Fig. 5) confirmed the result obtained by linear regression, i.e. a rising trend.

The 5-year forecasts provided by the ARIMA models and by linear regression pointed to an increase in both PI and PSD, with an increasingly early PSS. However, ARIMA improved forecasting by enabling the modelling of other features of the time series, such as cycle patterns.

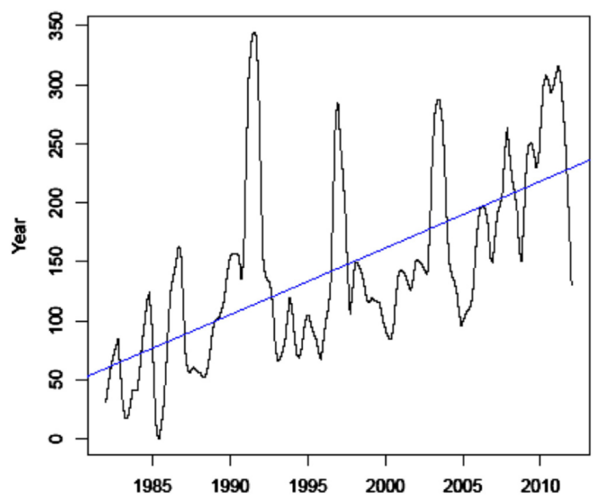

Fig. 3. Linear analysis of trend component of daily pollen time series.

Table 3
ARIMA summary. PSS, Pollen Season Start; PSD, Pollen Season Duration; PI, Pollen Index. Coef. (Coefficient), s.e. (Standard error), AR (Autoregressive), SAR (Seasonal Autoregressive), Log lik. (Log likelihood), AIC (Akaike Information Criterium), BIC (Bayesian information criterion).

Parameter	Model (PSS)		Model (PSD)		Model (PI)	
	Coef.	s.e.	Coef.	s.e.	Coef.	s.e.
AR1	−0.565	0.166	−0.884	0.196	−0.176	0.193
AR2			−0.476	0.192		
SAR1	−0.527	0.166	−0.764	0.135	−0.51	0.158
Model type	$(1,1,0)(1,1,0)_2$		$(2,1,0)(1,1,0)_2$		$(1,1,0)(1,1,0)_2$	
Log lik.	−109.67		−109		−290.37	
AIC	225.34		225.99		586.73	
BIC	229.23		231.17		590.62	

4. Discussion

The consequences of global climate change are evident from the observation of different climatic and biological manifestations which are directly or indirectly connected with the increase of the greenhouse effect (Bernstein et al., 2007). Many studies have confirmed the relationship between climate trends and various phenological phases in different plant and animal species (Sparks and Menzel, 2002; Beaubien and Freeland, 2000; Jaagus and Ahas, 2000; Kenda et al., 2000; Menzel and Sparks, 2006; Peñuelas et al., 2002; Chmielewski and Rötzer, 2001). Therefore, it can be assumed that a hypothetical future climate change will lead to changes in the range of the most sensitive species and a shift in the onset of different phenophases (Higgins and Richardson, 1999; Schwartz and Reiter, 2000). Research suggests that spring events in higher plants, leafing and flowering, have typically advanced, in some cases by several weeks (Bertin, 2008). Tree species are especially affected by climate warming, displaying in general an advance in flowering, leafing, and fruit ripening, particularly at high altitudes (Parmesan and Yohe, 2003).

In southern areas, the impact of climate change is being noticeable. According to the IPCC (Bernstein et al., 2007), Mediterranean ecosystems have certain vulnerabilities to climate change. Warmer and drier conditions will force species to shift. Land use, habitat fragmentation and intense human pressures will further limit natural adaptation responses, giving rise in many of these regions to a loss of biodiversity and of carbon sequestration services. The impact on plant response needs to be studied in greater depth. Most phenological research carried out in Europe provides few data on the Mediterranean region (i.e. Menzel, 2000; Vokou et al., 2012). The present findings confirm that olive phenology is being influenced by changes in climate. The annual pollen index is increasing, more pollen over more days, although the increase of Pollen Index could be partially explained by the increase of land surface devoted to olive cultivation suffered by the Córdoba province in the last years, around 20% from 1982 (SSYA, 2011). However, many scientific works have shown that temperature and water availability are highly correlated with the olive flowering intensity (Galán et al., 2001; Ribeiro et al., 2006a; Oteros et al., 2013b). In this sense, the increase of Pollen Index detected in our results can be also influenced by the increase of temperature during the study period. Regarding pollen season features, it is noticeable that the starting time is recorded earlier, pollen peak is being reached faster and the end is progressively later. Therefore pollen season is lengthening. These phenological changes can be attributed mainly to changes in temperature. The role of water availability on olive phenology is also important. Previous research has pointed out as water deficit can delay olive flowering phenology (Oteros et al., 2013c). Our results do not show this behaviour probably because the recorded rainfall is slightly increasing during the last 30 years, combined the fact of increasing temperatures (Galán et al., 2005; Ribeiro et al., 2006b; Orlandi et al., 2006; Aguilera et al.,

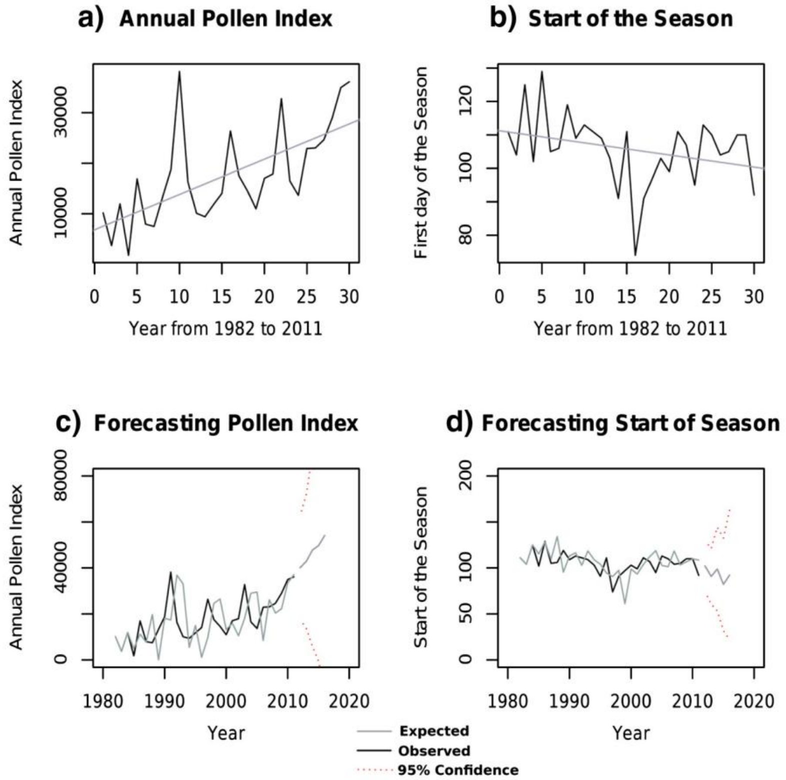

Fig. 4. Linear regression and ARIMA analyses for Pollen Index and the Start of Pollen Season. In a) and b) black line represents the real data and grey line represents the linear trend line in Linear regression analysis. The values forecasted by ARIMA models are represented in c) and d).

2013). Similar phenological changes, especially on flowering start, are also reported by other papers focussing on Spain and Italy (Galán et al., 2005; Bonofiglio et al., 2009, 2013; García-Mozo et al., 2010; Orlandi et al., 2013a, 2013b). Our results coincide in the fact of an olive flowering season advance although those related to the rest of phenological characteristics have been no possible to compare, due to the absence of works reporting this sort of results.

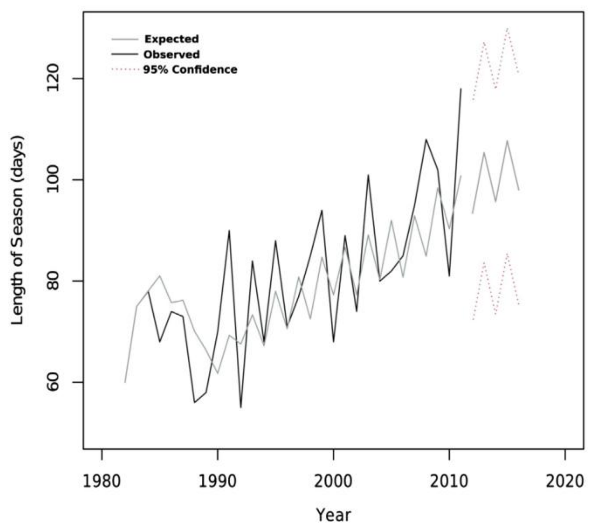

Fig. 5. Prediction of the length of the pollen season using ARIMA forecasting.

In anemophilous species of agricultural interest, like the olive, the study of flowering fluctuations provides valuable information on crop size (Galán et al., 2008). Changes in the length of the growing season influence the biochemical cycles of nitrogen, carbon and water, thereby affecting the productivity of natural terrestrial ecosystems and, in the case of the olive, agroecosystems (Ibáñez et al., 2010; Noormets et al., 2009). The Intergovernmental Panel on Climate Change (Bernstein et al., 2007) proposes several future climatic scenarios based on different estimations of humanity development and climate trends. The A1B emission scenario is the most accepted by scientific community, this future scenario assumes a rapid increase of greenhouse gas (having about 700 ppm of CO_2 concentration in 2100 year), increasing temperatures in the Mediterranean basin. More pollen grains produced by more flowers, due to climate warming and rising CO_2 levels; this could give rise to larger crops in future, on the other hand decreases in water availability could produce the opposite effect.

Shifts in olive flowering phenology involve agricultural economics both at the national and European community levels. Armed with improved knowledge of potential trends in olive flowering we can plan crop harvests and marketing, taking into account the influence of climate. Olive pollen research is also of particular interest for the general population, since pollen grains contain a number of different allergens that give rise to atopy and asthma in a large proportion of the population throughout the Mediterranean area (Barber et al., 2008).

Long-term trend analysis of phenological databases usually relies on linear regression techniques, although these are far from optimal because certain phenological features do not evolve linearly in time (i.e. Menzel, 2000; Dierenbach et al., 2013; Bartolini et al., 2013). Natural processes with a clear seasonal component, such as olive flowering, should be analysed using other kinds of statistical technique, including Seasonal-Trend Decomposition procedure based on Loess (Verbesselt et al., 2010; Pomati et al., 2012). The present study pioneers the use of this technique in aerobiological research. By removing the seasonal component, which shows variations in daily-pollen time series due to annual weather patterns, and by removing the remainder component, which shows variations due to random features mainly linked to the considerable variability of the Mediterranean climate, the real rising trend in pollen output over time becomes much more obvious.

Although some attempts have been made to use ARIMA analysis for predicting daily pollen counts (Rodriguez-Rajo et al, 2006), it was not used hitherto as a means of forecasting annual phenological patterns. Usually phenological modelling, both from field or aerobiological data, has traditionally relied on linear regression (García-Mozo et al., 2010; Bradley et al., 1999; Peñuelas et al., 2002). Our results indicate ARIMA as an appropriate method for modelling nonlinear and auto-correlated series. In our case, it has been a more accurate forecasting method than linear regression, because olive reproductive cycle has several cyclical features, including a bi-annual pattern in flowering intensity, that impede effective predictions using linear methods.

5. Conclusions

Pollen-season duration is increasing in South Spain due to the fact that both the Start and Peak dates are occurring earlier, whereas the End date is occurring later. The annual Pollen Index is increasing significantly, which has consequences for olive crops. The lengthening of the season combined with the increase in airborne pollen counts can play a negative effect on pollen-allergy sufferers in the Mediterranean area.

With regard to the statistical analysis performed, apart from the Regression Analysis, the Decomposition analysis by Loess confirms a clear rising trend in pollen output, when only the trend component of daily-pollen time series is analysed. ARIMA models are more effective than linear regression techniques for nonlinear time series.

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ORIGINAL PAPER

Heat accumulation period in the Mediterranean region: phenological response of the olive in different climate areas (Spain, Italy and Tunisia)

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Abstract The main characteristics of the heat accumulation period and the possible existence of different types of biological response to the environment in different populations of olive through the Mediterranean region have been evaluated. Chilling curves to determine the start date of the heat accumulation period were constructed and evaluated. The results allow us to conclude that the northern olive populations have the greatest heat requirements for the development of their floral buds, and they need a period of time longer than olives in others areas to completely satisfy their biothermic requirements. The olive trees located in the warmest winter areas have a faster transition from endogenous to exogenous inhibition once the peak of chilling is met, and they show more rapid floral development. The lower heat requirements are due to better adaptation to warmer regions. Both the threshold temperature and the peak of flowering date are closely related to latitude. Different types of biological responses of olives to the environment were found. The adaptive capacity shown by the olive tree should be considered as a useful tool with which to study the effects of global climatic change on agro-ecosystems.

Keywords Chilling · Flowering · Heat requirements · Olive · Plasticity · Threshold temperature

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Introduction

In the Mediterranean basin, the olive tree (*Olea europaea* L.) is one of the most widespread cultivated arboreal species. In this area, olive growing has a social and economic role that is of paramount importance, with olive fruit and oil being among the oldest and the most important of products.

The Mediterranean region accounts for approximately 98 % of the world production and 82 % of the world consumption of olive oil. Spain is the largest olive oil producing country, with 41.9 % of world production, followed by Italy (18.3 %), Greece (12.2 %) and Tunisia (5.9 %) (International Olive Council 2011). Together, these countries produce about 2,178,000 tonnes of olive oil, which represents 78 % of the world total olive oil production.

This region lies in a transition zone between the arid climate of North Africa and the temperate and rainy climate of central Europe. It is thus affected by interactions between mid-latitude and tropical processes, and is an area that is potentially vulnerable to the effects of climate change (Giorgi and Lionello 2008). Indeed, the Mediterranean region has been identified as one of the most prominent 'hot spots' in future climate-change projections (Giorgi 2006).

Climate affects practically all of the physiological processes throughout the life cycle of plants (Barranco et al. 2008; Osborne et al. 2000). Of all of the biological phases, flowering is the most critical for every fructiferous plant. Olive floral phenology is characterised by bud formation during summer, dormancy during autumn, budburst in late winter, and flowering in late spring (Fernández-Escobar et al. 1992; Galán et al. 2005). The release of floral bud dormancy occurs when olive trees have been exposed to a long enough period of chilling temperatures (Fernández-Escobar et al. 1992; Pinney and Polito 1990; Rallo and Martin 1991). Indeed, incomplete chilling can delay the release of floral bud dormancy, and thus delay the first flowering, which can result in an expanded flowering period (Barranco et al. 2008).

After budbreak, certain biothermic units are required for the development of the inflorescences (Chuine et al. 1998). Determination of the exact start date for olive heat accumulation has proven difficult, and remains lacking to date. Both the onset of the heat accumulation period and the temperature threshold for the amount of positive heat units might vary according to the climate of a determined geographical area. Changes in the temperature requirements might negatively affect the correct development of flower buds and the timing of olive flowering and, consequently, the evolution of the olive pollen season.

Many authors have used a combination of aerobiological and phenological information to analyse the flowering phase in anemophilous species, with pollen emission data being widely used as a well-proven tool for indirect evaluation of the flowering period (Galán et al. 2008; Jato et al. 2002; Orlandi et al. 2005, 2010). Nevertheless, the location of pollen monitoring stations, as well as both the topographical and the meteorological conditions should be considered (Aguilera and Ruiz Valenzuela 2009; Dimou 2012; Osborne et al. 2000).

Phenotypic plasticity is the ability of an individual organism to alter its physiology and/or morphology in response to changes in environmental conditions. This is particularly important in plants, where a sessile life-style requires them to deal with ambient conditions (Schlichting 1986). Moreover, Bradshaw (1965) reported that perennial species are more plastic than annual species, as the most obvious environmental changes that can affect a perennial species will be those connected with the seasons.

The climate of the Mediterranean region is characterised by a great diversity of features, which results in a variety of climate types and great spatial variability (Lionello et al. 2006). On this basis, the role of biothermic requirements related to the olive flowering period needs further evaluation.

The aim of the present study was to determine the start date of the period of heat accumulation and the optimal temperature threshold within the Mediterranean region across the large latitudinal gradient of Tunisia, Spain and Italy. At the same time, we evaluated the possible different types of plastic responses to the environment in these different populations of olive trees.

Materials and methods

Study area

The main characteristics of the olive growing areas considered in the present study are shown in Table 1. The latitudinal gradient through this Mediterranean region covers a large geographical area, from 43°05′N in Perugia, Italy, to 33°35′N in Zarzis, Tunisia. In terms of longitude, the study areas cover from 09°50′E in Menzel, Tunisia, to 04°45′W in Córdoba, Spain. The range of altitude is from 17 m a.s.l. in Zarzis, Tunisia, to 528 m a.s.l. in Jaén, Spain. The study period comprises from 13 years (1999–2011) to 20 years (1992–2001), depending on the year of onset of aerobiological monitoring in each area.

Bioclimatic data

To determine the optimal date for the onset of the heat accumulation period, chilling curves were constructed and evaluated for each year and study area on the base of daily chilling values. The chilling units were calculated by the Utah method (Richardson et al. 1974). This model assumes the presence of an optimum curve of the chilling effect, and it assigns a weighted value to each temperature data. A maximum chilling value at 5.2 °C was assigned, while a temperature below 0 °C and above the maximum threshold produces negative values (Orlandi et al. 2006). Of note, the function used to calculate the chilling units takes into account a threshold maximum temperature, which is more realistic for adaptation to the climate characteristics of each study area (Orlandi et al. 2006).

A maximum threshold temperature of 16 °C was used. If the temperature goes above this, then it is too warm for the olive to accumulate chilling. As soon as the temperature drops below this threshold temperature of 16 °C, different chilling values can be calculated on the base of the hourly temperatures considered by the Utah model. The 16 °C threshold was chosen because, considering all the study areas, it was the best temperature base for obtaining positive chilling units. Indeed, this was the lowest threshold temperature that allowed obtaining positive chilling units also in the southern Tunisian areas.

The chilling curves, derived by daily chilling values, were constructed as polynomial equations and were used for estimation of the heat accumulation start date (start_ht) for each year and study area. Eight heat accumulation start dates were tested: the peak of chilling (peak_ch) as the day of the year with the maximum accumulation of positive chilling units, and the following days with the decline in the cumulative chilling percentages of 1 %, 3 %, 5 %, 10 %, 15 %, 20 % and 25 % (relative to the peak of chilling).

The heat amounts are expressed as growing degree days (GDDs, °C-day). The GDD values were calculated using the

Table 1 Biogeographical characteristics of the olive growing study areas in Italy, Spain and Tunisia

Site	Coordinates	Altitude (m a.s.l.)	Mean annual temperature (°C)	Annual total precipitation (mm)	Studied period
Italy					
Perugia (PG)	43°05′N,12°30′E	450	13.6	850	1992–2011
Brindisi (BR)	40°55′N,17°01′E	230	16.6	573	1999–2011
Spain					
Córdoba (CO)	37°50′N,04°45′W	123	18.0	674	1992–2011
Jaén (JN)	37°47′N,03°46′W	528	16.9	578	1993–2011
Tunisia					
Menzel (ME)	35°25′N,09°50′E	160	20.9	317	1993–2011
Zarzis (ZR)	33°35′N,11°01′E	17	21.0	192	1993–2011

method proposed by Arnold (1960), based on the maximum and minimum daily temperatures. This model assumes a temperature base below which biothermic accumulation stops or is reduced to the lowest terms. To determine the most appropriate temperature base for the heat accumulation for each area, a wide range of 25 threshold temperatures from 1 °C to 25 °C were tested.

To interpret the relationship between the heat accumulation period and the olive flowering phase, aerobiological databases were used (International Association for Aerobiology Newsletter 2011). Sampling was carried out by the volumetric method, which is based on the possibility of capturing pollen and other biological particles present in the atmosphere using the principle of depression impact. Aerobiological sampling techniques provide data on daily pollen content in the air that reflect the process of flower opening in the olive growing areas around a monitoring station. Moreover, this kind of sampling reduces the subjectivity in interpretation of the flowering period. Considering that the monitoring traps were located inside or very near to the olive groves, the peak day of the pollen season, that is, the day with the maximum olive pollen concentration, was used as the full flowering date (peak of flowering; peak_fl) (Galán et al. 2008; Orlandi et al. 2010; Osborne et al. 2000).

The different GDD amounts were calculated from the start dates above to the peak of flowering, for each year and study site. The root mean square errors (RMSEs) were calculated and plotted. The most accurate start heat accumulation date and the best threshold temperature were selected for each area, which took into account the lowest RMSE mean value over the study period.

Three variables related to the heat accumulation period were calculated: (1) the length (in days) of the heat accumulation period (length ht period), which was calculated as the time difference between the best heat accumulation start date and the peak of flowering date; (2) the time period between the peak of chilling date and the heat period start date (peak_ch/start_ht); and (3) the duration of the period between the peak of chilling date and the peak of flowering date (peak_ch/peak_fl).

Maximum and minimum daily temperatures were obtained from the weather stations nearest to the study monitoring units, i.e. Network stations of the National Council of Agricultural Research (CRA-Cma) for Italy, the Agroclimatic Station Network of the Andalusian *Consejería de Agricultura y Pesca* for Spain and the National Meteorological Centre for Tunisia.

Statistical analysis

To define the degree of the relationship between heat accumulation period characteristics and olive reproductive phase, Pearson's parametric correlation analysis was performed, in relation to data normality. A 95 % confidence level was used. Only significant absolute coefficient values ≥0.50 were considered. Similarly, the correlation coefficients between interrelated variables were not considered; i.e. a variable calculated by the combination of the other two variables (e.g. the length of the heat accumulation period is the difference between the peak of flowering date and the start heat date).

Linear regression analyses were also performed according to the latitude, altitude and bioclimatic parameters, to establish the highest statistical relationship of dependence for olive flowering.

Variance analysis (one-way ANOVA) was carried out to determine whether the differences in the heat accumulation periods were induced by variable geographic environmental conditions. When there were significant differences, a post-*hoc* Tukey test was performed. This comparative analysis between the study areas allowed evaluation of the possible different types of biological response of the olive trees to the environment.

Statistica 7.0 software was used for all statistical analyses.

Results

The mean RMSE values obtained relating to both the different heat accumulation start dates and all of the threshold temperatures tested for each study area are shown in Fig. 1.

The best start date for the onset of the heat accumulation period in the olive growing areas of Perugia and Brindisi

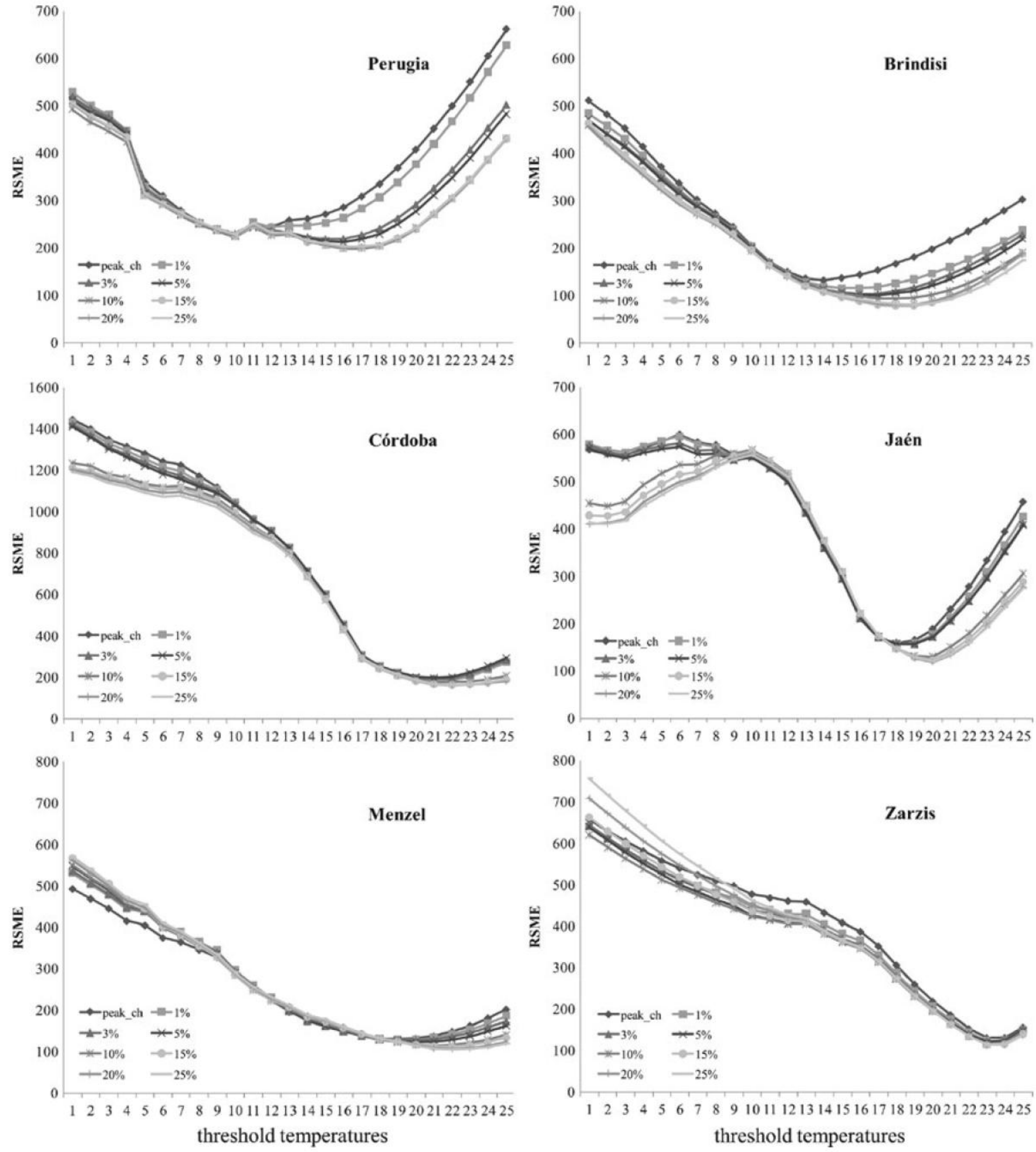


Fig. 1 Mean root mean square error (RMSE) values for the different heat accumulation start dates and the different threshold temperatures evaluated for each study site. *Peak_ch* Peak of chilling date

was the day with a 10 % cumulative chilling percentage decline in comparison to the peak of chilling. In Córdoba, 15 % was obtained as the best heat accumulation start date, while 20 % was obtained for the Jaén site. In this latter area, a 20 % cumulative chilling percentage decline in comparison to the peak of chilling was the best heat accumulation start date for both the Menzel and Zarzis olive-growing areas.

Different threshold temperatures were obtained across the study areas, with values that ranged from 16 °C in Perugia to 23 °C in Zarzis (Table 2). Two simple linear regression analyses that tested for relationships between the threshold temperature and the main geographical characteristics are shown in Fig. 2. A significant and strong negative relationship (R^2=0.91) was found between latitude and threshold temperature. This relationship was lowest in terms of the altitude (R^2=0.49).

The average dates of peak of chilling for each of the study areas are given in Table 2. This parameter varied geographically (F=10.777; P<0.001). The average peak of chilling in the Perugia, Brindisi, Menzel and Zarzis olive growing areas was between 18 and 22 January, while in Córdoba and Jaén, this average date occurred as 11 January.

Table 2 Average values of the main characteristics of the heat accumulation period in each study area. *Peak_ch* Peak of chilling date, *Start_ht* heat accumulation start date, *Peak_fl* peak of flowering date, *GDD* growing degree days amounts

Study area	Peak_ch	Start_ht	Peak_fl	Threshold	GDD	Peak_ch/ Start_ht	Length heat period	Peak_ch/ Peak_fl
Perugia	18±7	46±10	158±6	16	200±47	28	112	140
Brindisi	20±6	41±8	141±4	18	123±30	21	100	121
Córdoba	11±6	37±8	133±13	22	119±38	26	96	122
Jaén	11±9	39±9	134±7	20	114±28	28	95	123
Menzel	22±7	36±7	119±8	22	81±25	14	83	97
Zarzis	22±6	32±8	114±9	23	83±27	10	82	92

It is noteworthy that, although similar peak chilling dates were recorded in the Italian and Tunisian areas, the chilling amounts were clearly different. The average chilling accumulation from the first day in which positive chilling units were detected to the peak of chilling date was 1,246 chilling units in Perugia and 1,034 chilling units in Brindisi, which are notably higher than the 130 and 27 chilling units recorded in Menzel and Zarzis, respectively. In the Spanish areas, this accumulation value was 606 chilling units for Córdoba and 767 chilling units for Jaén.

The heat accumulation start dates were significantly different between these olive growing areas (F=5.825; P=0.001). Two groups were differentiated (Fig. 3). The average heat start date in Perugia was 15 February (Julian day 46). However, in Brindisi, Córdoba, Jaén, Menzel and Zarzis, the average heat accumulation start date was from 1 to 10 February (Julian days 32 to 41).

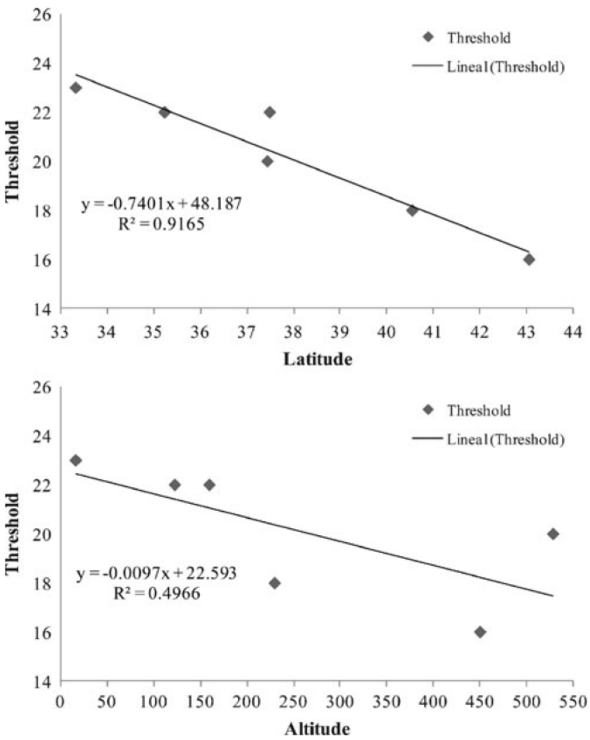


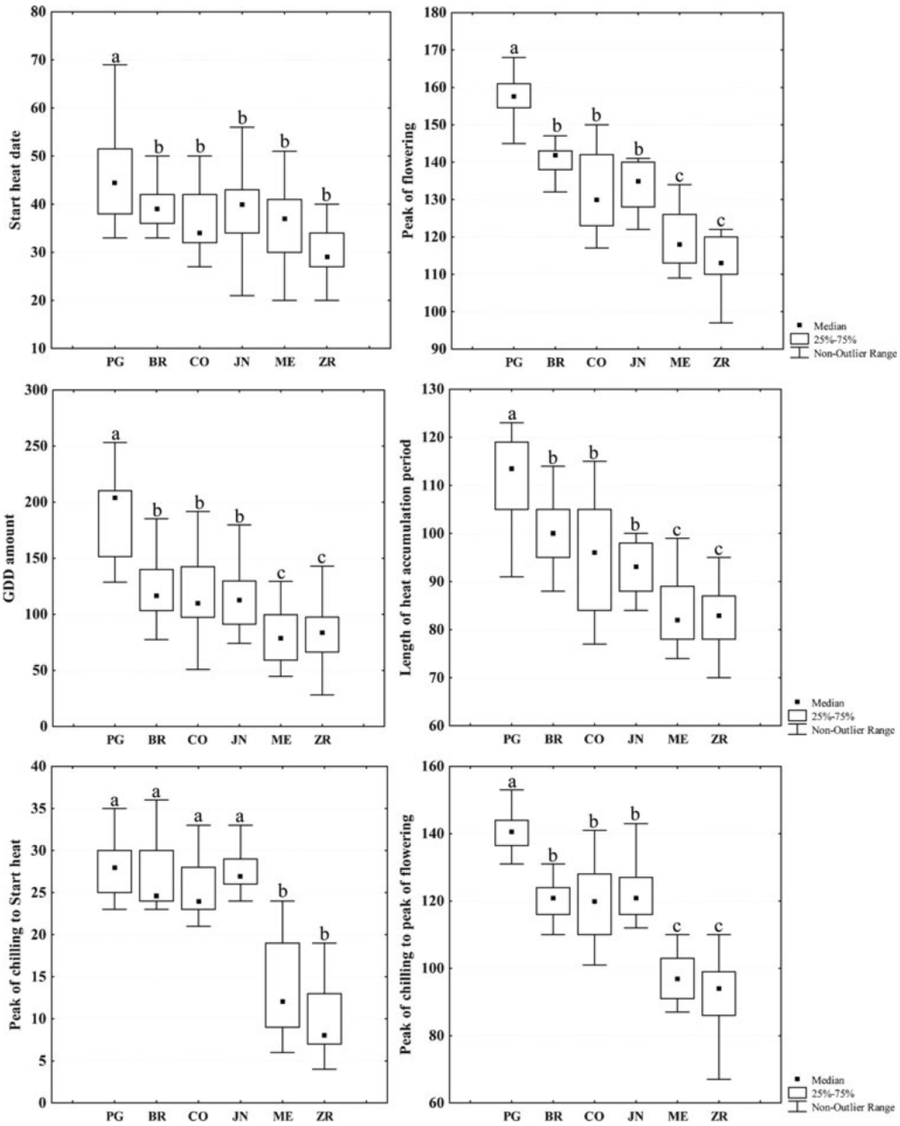
Fig. 2 Linear equations and fitting of the regression analyses between latitude, altitude and threshold temperatures

For the average flowering peak date, significant differences between these sites were found (F=62.030; P=0.001). Here, three groups were observed. The average peak of flowering date in the olive growing areas of Menzel and Zarzis were 24 and 29 April (Julian days 114 and 119), respectively, followed by the average flowering peak dates recorded in Brindisi, Córdoba and Jaén, which were between 14 and 21 May (Julian days 134 to 141). For the Perugia site, the average peak of flowering was 7 June (Julian day 158). The linear regression analysis between the peak of flowering date and the latitude is shown in Fig. 4. A highly significant positive relationship between latitude and peak of flowering was obtained (R^2=0.97). The same type of relationship was seen for the altitude, although with a lower R^2 (0.47).

Significant differences between study areas were recorded for both the length of the heat accumulation period (F=23.630; P<0.001) and the GDD amounts (F=31.998; P=0.001) and, as above, three groups were observed. Perugia showed the highest length of heat accumulation period and the highest GDD amounts recorded, with means of 112 days and 200 GDD (threshold temperature, 16 °C), respectively. In the Brindisi, Córdoba and Jaén olive growing areas, the length of the heat accumulation period was from 95 to 100 days, and a mean of 119 GDD (threshold temperature, 18 °C–22 °C) was recorded. Menzel and Zarzis showed the lowest values, with a mean length of heat accumulation period of 83 days, and a mean of 82 GDD (threshold temperature, 22 °C–23 °C). The correlation analysis carried out to test the relationship between latitude, altitude and GDD amounts showed a highly significant positive relationship between latitude and GDD amount (r=0.71). However, this relationship was lower regard to the altitude (r=0.50).

The time periods between the peak of chilling date and both the heat period start date and the peak of flowering date were also analysed. For the first of these, two groups with clear differences were recorded (F=47.710; P=0.001). The study areas of Perugia, Brindisi, Córdoba and Jaén showed

Fig. 3 *Box-plots* of the different parameters studied. *PG* Perugia, *BR* Brindisi, *CO* Córdoba, *JN* Jaén, *ME* Menzel, *ZR* Zarzis. Different letters indicate statistically different values ($P \leq 0.05$)



the highest mean values, from 21 to 28 days, whereas in the Menzel and Zarzis olive areas these were 14 days and 10 days, respectively. For the second parameter, as the time period from the peak of chilling to the peak of flowering, three significantly different groups were observed ($F=58.060$; $P=0.001$). The mean length of this time period in Menzel and Zarzis was 95 days, follow by 122 days in Brindisi, Córdoba and Jaén. The highest value was observed in Perugia, at 140 days.

Correlation analysis was performed for the relationships between the heat accumulation period characteristics in each study area. In general terms, the start date of the heat accumulation period correlated significantly and positively with the peak chilling date (Perugia $r=0.96$; Brindisi $r=0.99$; Jaén $r=0.93$; Menzel $r=0.76$; Zarzis $r=0.84$). Non-significant coefficients were obtained for the other parameters considered in this analysis. The GDD amounts showed significant and positive correlation coefficients in some areas. The time period between the peak of chilling and the start heat accumulation date had a positive influence on the GDD amounts in the olive groves of Perugia ($r=0.51$) and Jaén ($r=0.70$). Moreover, the peak of flowering in Córdoba correlated significantly, although with a lower coefficient, with the GDD amounts ($r=0.51$). The time period between the peak of chilling and the start heat accumulation date also had a positive influence on the peak of flowering. This variable correlated significantly in the Perugia ($r=0.53$), Córdoba ($r=0.55$) and Jaén ($r=0.59$) olive-growing areas. The peak of chilling

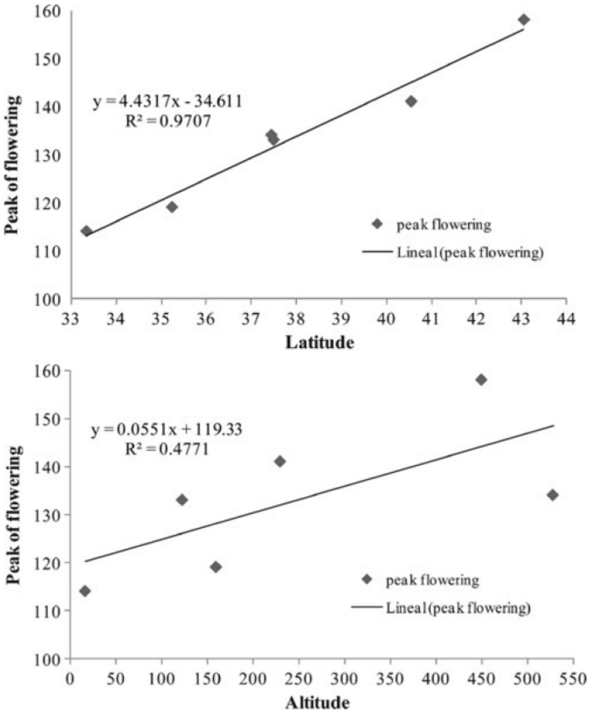


Fig. 4 Linear equations and fitting of the regression analyses between latitude, altitude and average peak of flowering dates

date correlated positively with the peak of flowering in the Menzel area (r=0.62).

Discussion

The specific moment for the onset of the olive heat accumulation period is difficult to determine and has essentially remained unknown. Flower buds that are ready to begin the first phase of morphological development require a certain thermal contribution to continue to develop after their latent phase (Anderson et al. 2005; Foley et al. 2009; Orlandi et al. 2002). Numerous studies, adopting methodologies and threshold temperatures of great heterogeneity, have investigated the biothermic requirements regarding the biological development of the olive in different areas (Alba and de la Guardia 1998; Bonofiglio et al. 2008; Galán et al. 2005). The GDD is a commonly used thermal time method of widespread acceptance, but agreement in the method for evaluating the best threshold temperatures is currently lacking (Ruml et al. 2010; Snyder et al. 1999; Yang et al. 1995).

The threshold temperatures obtained in this research field differ from those provided for the olive tree in previous studies (Galán et al. 2005; Martins et al. 2012; Orlandi et al. 2006). The base temperatures reported by these authors ranged between 5 °C and 12.5 °C, values lower than those are shown here. This fact can be considered to depend mainly on methodology used both to determine the onset of the heat amount and to statistically evaluate the best GDD threshold temperatures. Moreover, there are other factors that should be considered. On the one hand, the lower threshold or base temperature may vary between geographical areas (Črepinšek et al. 2006). In this sense we have observed that the threshold temperatures are related closely to latitude. Threshold values decrease proportionally as the latitude increases, as these values are lower in olive groves at higher latitudes. The same relationship was seen for altitude, although at a lower intensity. Furthermore, and according to several studies, it should not be surprising that the temperature affects both the dormancy induction and flowering time, and that, in the northern cooler areas, the olive trees consider as heat lower temperatures than in the southern areas (Edwards et al. 2006; Foley et al. 2009; Ramos et al. 2005). On the other hand, threshold temperatures could differ between species and also between cultivars within a species (Martins et al. 2012; Ruml et al. 2010). It would be interesting to take this point of view into account in future studies in the Mediterranean region considering different olive cultivars located at the same latitude to avoid geographical variability.

The construction and evaluation of chilling curves to determine the start date of the heat accumulation period was successful. The peak of chilling, i.e. the day of the year with the maximum accumulation of positive chilling units, has not been considered as the start date of the heat accumulation in any olive growing area. This result is particularly important because it could indicate that, upon arriving at the peak chilling day, the chilling requirements may have not been met, and more days of chilling would be needed to break the endodormancy phase (Lang 1987).

Once the chilling requirements are met, crown buds remain in temporary suspension, with their visible growth state being controlled by environmental factors (ecodormancy) (Lang 1987). We have observed that, from the peak of chilling, a certain number of days are needed to begin the heat accumulation period, which, as seen above, might be related closely to the transition from endodormancy to ecodormancy. According to Doorenbos (1953), the transition from one phase of dormancy to another does not show morphological evidence that would allow an unequivocal classification. Moreover, De la Rosa et al. (2000) reported that active growth and development starts as soon as buds that have completed chilling are placed under favourable conditions for budbreak. In this sense, the results of the present study provide a good approximation. Here, in all study areas, the range from 10 % to 20 % cumulative chilling percentage decline in comparison to the peak of chilling provided the best start date for the onset of the heat accumulation period.

On this basis, two types of biological responses of olives to the environment were seen. In the Italian and Spanish olive-growing areas, the time period between the peak of chilling and the heat accumulation start date was 2 weeks longer than in the Tunisian areas. This finding might indicate that olive trees located in the warmest winter areas have a faster transition from endogenous to exogenous inhibition once the peak of chilling is met, which is consequently related to their adaptive capacity to the environment concerned. Several studies have reported that, for the olive, low temperatures are needed only to break dormancy in previously initiated buds, as occurs in other fruit-tree species (Fernández-Escobar et al. 1992; Pinney and Polito 1990; Rallo and Martin 1991). However, our data indicate that a delay in the peak of the olive flowering date can be caused by a long transition between the end of internal dormancy and the onset of ecodormancy. This was observed in the Italian and Spanish areas, but not in the Tunisian areas, where there is a more rapid transition between the dormancy phases.

In the different study areas from the coldest to the warmest, the chilling requirements for successive flowering development can be considered as adequately fulfilled even if the differences were elevated in areas of central Italy in comparison with those in south Tunisia. Until now, the lowest winter chilling amounts recorded in north African areas were also sufficient to regulate the olive dormancy phenomenon but in the future these might no longer be adequate. In this sense, genetic selection to obtain new olive cultivars with commercial quality and low chilling requirement, which is a heritable character (Barranco et al. 2008), could be a convenient agronomical practice, as could the applications of rest-breaking agents, developed to avoid or reduce the negative consequences of an insufficient chilling accumulation.

The onset of the heat accumulation period usually occurs in the first two weeks of February, although in Perugia there was a delay of 9 days compared to the other areas studied. This date is positively related to the occurrence of the peak of chilling. It is noteworthy that, once the heat accumulation period begins, different biological responses can be observed with respect to subsequent floral development.

The olive groves located in the northern areas (e.g. the Perugia site) need a longer period of time than the other areas to completely satisfy their biothermic requirements, with these being notably higher than those in the warmer areas. The lowest heat requirements were observed in the southern areas (e.g. the Tunisian sites), where there was a mean difference of 59 % with respect to the biothermic requirements in the northern olive-growing areas. These low heat requirements are due to better adaptation to warmer regions.

The same phenomenon was seen for the peak of flowering. The day on which most of the tree canopy flowers are open, and consequently the main flowering date, is influenced strongly by latitude. Thus, olive trees located in the Tunisian areas were the first to complete flower development and to begin their flowering period. Both the GDD amounts and the peak of flowering date increase progressively with latitude. This is in agreement with Bonofiglio et al. (2008), who reported homogeneity in plant behaviour as a function of latitude.

Another particularly interesting result is that the period of time between the day with the maximum accumulation of chilling and the day with the maximum olive pollen emission to the atmosphere was notably shorter in the southern areas than in olive groves situated at intermediate and high latitudes. It is important to consider that both the metabolic activity and the photosynthetic activity of the olive are maximal across an optimal range of temperature between 15 °C and 30 °C (Krueger 1994). According to this latter author, respiration is catalysed by enzymes that are temperature sensitive. In the temperature range of 10 °C–30 °C, respiration shows a positive trend line, doubling for each 10 °C increase in temperature. However, at higher temperatures, the enzymes can be denatured. In addition, excessive temperatures of around 30 °C–35 °C can be a limiting factor for olive reproductive success (Barranco et al. 2008; Cuevas et al. 1994; Martin et al. 1994). High temperatures when olive flower buds are developing, especially if these temperatures are accompanied by dry, windy conditions, can have a detrimental effect on subsequent flowering, pollination and fruit set (Martin et al. 1994). Moreover, the olive pollen tube growth is maximal at 25 °C, while temperatures higher than this value show a negative influence (Cuevas et al. 1994; Koubouris et al. 2009). Hence, and according to these authors, excessive temperatures can negatively influence several reproductive parameters such as flower development, pollen tube growth, pollen viability or the pollination process, and, consequently, subsequent fertilisation and production yield.

For this reason, olive trees situated in southern areas develop all of this process in a shorter time, through adapting their physiology in response to the very high temperatures, probably as a defence mechanism. On the other hand, the olive has also developed environmental control in more northern olive-production areas, in this case to avoid injury due to the potential occurrence of freezing during the first spring weeks (i.e. end of March to beginning of April). In these areas, the higher heat requirement to completely develop floral buds assures that plants reach their flowering period during the second half of May to the beginning of June, when the warmer season is definitely established, and before very high temperatures can stress the flower apparatus and pollen viability during the fertilisation processes (Aguilera and Ruiz Valenzuela 2012; Bonofiglio et al. 2008).

In the future, a substantial drying and warming of the Mediterranean region is expected, with a decrease in precipitation of around 25 % to 30 %, and warming that might exceed 4 °C–5 °C (Giorgi and Lionello 2008). In this regard, a decrease in the biothermic requirements and a more rapid transition from endodormancy to ecodormancy can be hypothesized in future scenarios, and consequently, the more rapid development of the floral buds, which will lead to earlier flowering peak dates in the southern areas in particular. Therefore, the adaptive capacity shown by the olive tree can be considered as a useful tool with which to study the effects of global climatic change on agro-ecosystems located in this especially vulnerable region.

Conclusions

The data presented herein allow us to conclude that northern olive populations have the greatest heat requirements for development of their floral buds, and that they need a longer period of time than olives in other areas to completely satisfy their biothermic requirements. The olive trees located in the warmest winter areas have a faster transition from endogenous to exogenous inhibition once the peak of chilling is met, and they show more rapid floral development. Different types of biological response by different populations of olive trees occur over this large Mediterranean area. These differences can be shown to arise from the adaptive biological responses of the olive to the environment. The present study provides us with interesting conclusions and a basis for further investigations that will make it possible to interpret the biological responses of the olive to future climate change.

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Quality control in bio-monitoring networks, Spanish Aerobiology Network

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HIGHLIGHTS

► A proficiency testing in the Spanish Aerobiology Network has been developed.
► The quality of all data has been evaluated, to give an overall assessment of the network.
► The individual performance of staffs has been evaluated and the source of errors committed has been identified.
► In general, technical staff performed well.

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ABSTRACT

Several of the airborne biological particles, such as pollen grains and fungal spores, are known to generate human health problems including allergies and infections. A number of aerobiologists have focused their research on these airborne particles. The Spanish Aerobiology Network (REA) was set up in 1992, and since then dozens of research groups have worked on a range of related topics, including the standardization of study methods and the quality control of data generated by this network.

In 2010, the REA started work on an inter-laboratory survey for proficiency testing purposes. The main goal of the study reported in the present paper was to determine the performance of technicians in the REA network using an analytical method that could be implemented by other bio-monitoring networks worldwide. The results recorded by each technician were compared with the scores obtained for a bounded mean of all results. The performance of each technician was expressed in terms of the relative error made in counting each of several pollen types.

The method developed and implemented here proved appropriate for proficiency testing in interlaboratory studies involving bio-monitoring networks, and enabled the source of data quality problems to be pinpointed. The test revealed a variation coefficient of 10%. The relative error was significant for 3.5% of observations.

In overall terms, the REA staff performed well, in accordance with the REA Management and Quality Manual. These findings serve to guarantee the quality of the data obtained, which can reliably be used for research purposes and published in the media in order to help prevent pollen-related health problems.

1. Introduction

Aerobiology studies the passive transport of microorganisms and biological particulate matter through the air. It has been widely demonstrated that certain biological particles present in the atmosphere, such as pollen grains and fungal spores, give rise to human health problems including allergies and infections. Moreover, human activity, such as changes in land use or in atmospheric emissions, is changing the biological effects of these particles on human health (Foley et al., 2005; Bartra et al., 2007). A number of recent papers have sought to chart changes in the content and behavior of airborne biological particles due to global climate change (Breton et al., 2006; Zhong et al., 2006; Docampo et al., 2011; Zhang et al., 2012; Ziello et al., 2012). Monitoring of the atmosphere is of crucial importance in ensuring the availability of large data series that enable to study the changes caused by human activities over time, and a number of aerobiology networks have been set up with this purpose in mind. Since the concentration of airborne biological particles, such as pollen grains, is closely linked to the incidence of adverse health reactions, there is a clear need to obtain databases to develop forecasting models, likely to further the adoption of preventive measures. The reliability of the data in databases is necessary to ensure the robustness of results obtained from them (Ferretti, 2011). For this purpose aerobiology networks require a standardized methodology and also an effective internal quality control through the implementation of quality programs (Quevauviller et al., 1996; Araujo et al., 2010).

The Spanish Aerobiology Network (REA) was founded in 1992, and since then the outset has belonged to the European Aeroallergen Network/European Pollen Information (EAN/EPI). From the beginning, the main goal of this network has been to use a standardized methodology to generate information regarding airborne concentrations of biological particles – especially pollen grains and fungal spores – at a national level. Research in this field has focused on a range of methodological issues, including the effective comparison of data obtained at

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different altitudes (Galan et al., 1995; Alcazar et al., 1999a, 1999b; Velasco-Jiménez et al., 2012), the sampling media used (Tormo et al., 1996; Galán and Dominguez Vilches, 1997; Comtois et al., 1999; Carvalho et al., 2008), the counting method employed (Cariñanos et al., 2000; Sikoparija et al., 2011; Cotos-Yáñez et al., 2012), the quality control tools applied (Docampo et al., 2009), and the definition of the pollen season (Jato et al., 2006). This research and during our long time acquired experience, led to the publication of the REA Management and Quality Manual (Galán et al., 2007) (from now REA-MQM), taking into account the EAN/EPI minimum requirements in the Methodology for Routinely Performed Monitoring of Airborne Pollen (Jäger, 1995).

Regarding quality control, different papers have been focused on detecting the source of errors. In order to improve the error detection on aerobiology, management and resolution, it is necessary to recognize the various sources of error in aerobiological data. For this purpose, we have performed below a description of the possible sources of error in these data, summarized in Fig. 1. Comtois et al. (1999) provided a preliminary overview of the types of errors affecting aerobiological data; their classification was enlarged here via the inclusion of other error sources. As Fig. 1 shows, aerobiological data can contain both random errors (errors) and systematic errors (bias). *Bias* may be defined as a non-random error which is usually unidirectional (systematic). A number of authors have sought to detect the bias caused by the counting method used (Gottardini et al., 2009), but bias may also be linked to many other methodological issues (Comtois and Mandrioli, 1997; Galán and Dominguez Vilches, 1997; Alcazar et al., 1999b). By contrast, *random errors* are the representation of the uncertainties of the natural world. Two types of random errors can be distinguished: those that arise by chance because of the working protocol, and instrument errors attributable to human failures. Random errors occurring as a result of the *working protocol* have been analyzed by several researchers, who have focused on a comparison of different counting techniques (Käpylä and Penttintn, 1981; Tormo et al., 1996; Comtois et al., 1999; Cariñanos et al., 2000; Sikoparija et al., 2011; Cotos-Yáñez et al., 2012). *Instrumental errors* arise from human failures in carrying out aerobiological analysis. Instrumental errors can be in turn divided into two kinds: *technician errors*, committed by operators in *counting* and *identifying* pollen grains, and *mathematical errors* arising from the data-correction method used to estimate real airborne particle.

Few research papers have specifically addressed quality control in inter-sampler comparisons carried out for proficiency testing purposes, this being the only way to detect instrumental errors. Berti et al. (2009) analyzed the precision and accuracy of various technicians using concentrations found by experts as true sample values. Berti et al. (2009) analyzed the total concentration of pollen grains and the total number of taxa identified in samples. Nevertheless, the distinction between pollen types is essential for detecting the source of instrumental error and subsequently applying effective corrective measures. Another study, outlined by Pedersen and Moseholm (1993) also stratified total pollen grains by taxa, but sought to investigate the reproducibility of pollen counts; since its main aim was not to study proficiency, it did not focus on the source of instrumental errors.

The main aim of the study was to evaluate the quality of all data and to give an overall assessment of the Spanish Aerobiology Network. The second aim of the present study was to perform a proficiency test to assess the performance of technicians and lay the groundwork for future research. And the third objective was to identify the source of any errors identified; to this end, a procedure has been developed to identify the source of the instrumental error and, therefore, to improve plans to remedy that error. All these goals allow us facilitate corrective actions and increase the effectiveness of REA quality improvement plans.

2. Material and methods

The *Spanish Aerobiology Network* (REA) has been involved in an ongoing quality-control program (QC). Given that the performance of aerobiological technicians is a crucial element in ensuring data quality, in 2010 the REA embarked upon an interlaboratory proficiency-testing study. The University of Córdoba working group, as the REA Coordinator Center, is providing scientific support for the inter-laboratory study, which follows IUPAC (Thompson et al., 2006) and ISO recommendations in order to assess the performance of each technician (ISO 17043). Standards on chemical testing have been adapted to specific characteristics of aerobiological data. The performance of each staff member is expressed as the relative error in pollen counts and the overall quality will be indicated by the variation coefficient and the number of cases in which the relative error is higher than acceptable. The relative error and the variation coefficient are obtained from calculation of several summary statistics contained in ISO instructions (ISO 13528).

A first step was focused on checking which REA members were interested in participating in the external QC exercise. 25 technicians belonging to 17 different REA research groups: Badajoz, Bilbao, Cartagena, Córdoba, Granada, León, Madrid, Málaga, Mallorca, Orense, Oviedo, San Sebastián, Santiago, Sevilla, Toledo, Vitoria and Zaragoza, have been involved on this proposal. Trying to study the most representative pollen types, three slides, from winter, spring and summer, obtained by Hirst type sampler into different geographical areas, have been distributed to the 25 participants. All technicians were instructed to analyze the same pollen types: *Amaranthaceae*, *Alnus*, *Fraxinus*, *Cupressaceae*, *Pinus*, *Populus*, *Platanus*, *Betula*, *Quercus*, *Morus*, *Urtica*, *Olea*, *Poaceae*, *Castanea*, *Rumex*, *Plantago* and total pollen count, following REA-MQM: counting method consists of 4 continuous horizontal sweeps over the whole slide with a 40×10 lens. This gives a subsample accounting for 12–13% of the total surface, depending on the microscopic field size at that magnification, which may vary depending on the microscope model. A percentage over 10% of analyzed surface has been recommended by the EAN/EPI in recent minimum recommendations. Data were expressed as average daily pollen counts per cubic meter of air.

Each pollen type on each slide was analyzed separately. Statistically speaking, each slide was considered as a set of P populations, where P was the number of pollen types analyzed. These were "special populations" with size $N = 1$, standard deviation $\sigma = 0$, in which the population mean (μ) was represented by the only value, that is the real

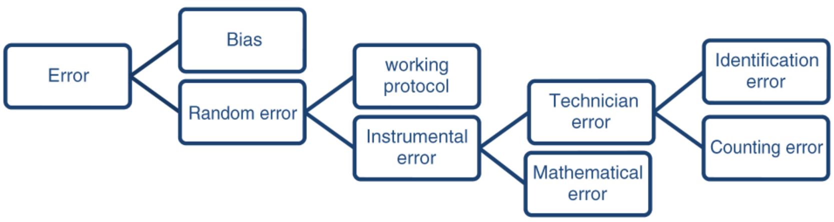

Fig. 1. Possible sources of error in aerobiological data.

concentration of pollen grains in the slide (unknown to us). A sampling on each population was carried out, being the size of each sample $n=25$ elements, where each element represented the concentrations obtained by each technician staff.

To know the staff proficiency, we need to compare the counts of each staff with the unknown real value (μ). An approximation of this was used here as a reference for calculating the relative error committed by each technician. This parameter is commonly termed the “Assigned Value” (X). The Assigned Value depends directly on the sample mean ($\overline{X}$), but with several modifications to make it closer to the population mean. It is important to understand the differences between three concepts: population mean (μ), sample mean ($\overline{X}$) and Assigned Value (X).

To meet our three objectives (assessing the overall quality of the data, the individual staff proficiency and finding for the source of errors detected), we have followed four major steps: 1) analyze data normality and outliers; 2) calculate summary parameters; 3) calculate and evaluate errors; and 4) investigate the source of errors. The individual proficiency is evaluated in step 3 and causes of errors are analyzed in step 4. Finally, to evaluate the overall quality of the data using the variation coefficient (calculated in step 2) and the percentage of error (calculated in step 3). All this procedure allows an increase in the data quality of the network and makes useful recommendations for other bio-monitoring networks in the world.

2.1. Normality and outliers

2.1.1. Normality

Results deriving from data whose distribution is non-normal should be viewed off course with caution, since many statistical procedures are only applicable to random samples from populations with a Gaussian distribution in order for results to be solid (Elveback et al., 1970; Limpert et al., 2008). Even the outcome of the simplest parameter “mean”, which should be a good estimate of the true value, depends strongly on the type of data distribution. If any sample doesn't follow a normal distribution, we cannot estimate the population mean. In this case we will not use the involved pollen taxa given the impossibility of knowing the real concentration in the slide. The normality of data distribution has been checked using the Lilliefors test (Starink and Visser, 2010).

2.1.2. Outliers

Statistical outliers may affect statistical parameters, and for that reason were eliminated here. Many standard outlier tests are not able to detect gross outliers, and a number of methods have been suggested for their detection (e.g. Dixon test, Grubb's test, and Huber's test). In the present study, the ISO recommended confidence levels were used, i.e. 95% for outliers classed as “stranglers” and 99% for those classed as “statistical outliers” (ISO 5725-2).

To calculate the Assigned Values for samples, z-scores were calculated for the central 95% of data, thus also eliminating outliers. The z-score of a raw score χ_i is given in formula (1), where $\overline{X}$ is the mean of the sample and S is the standard deviation of the sample. Values above 1.96 or below −1.96 lay outside the central 95% and were not taken into account in calculating the Assigned Values.

$$\frac{Z=\chi_i-\overline{X}}{S} \tag{1}$$

To construct Assigned Values, only results with a z-score of less than 1.96 were taken into account and an outlier should also meet the following condition: $\chi_i-\overline{X}>|10|$, where χ_i is a raw score and $\overline{X}$ is the mean of the sample. This was because z-score values are strongly influenced by the sample mean: if the sample mean is very low, the z-score cannot identify the true outliers. Moreover, samples with a low mean are unlikely to fit into a normal distribution (Altman and Bland, 1995).

2.2. Summary parameters

Summary statistical parameters were calculated after rejection of non-valid results, and constructed for each pollen type: one summary parameter summarized the central value of data (*mean*), and another summarized data dispersion (*standard deviation*), confidence intervals and variation coefficients. Summary statistics were obtained following ISO recommendations (ISO 13528).

2.2.1. Assigned Value

“Assigned Value” (X) for each population was calculated using the average of the data contained within the central 95% of values for the sample. The sample was bounded by calculating z-scores as indicate d in Section 2.1.2. X is an approximation of the population mean (μ) that is also the true value of pollen concentration.

2.2.2. Standard deviation for proficiency

The standard deviation (S) of the data with a 95% probability of belonging to the population was used as a summary parameter for data dispersion. This parameter was termed “standard deviation for proficiency” (S'). The procedure outlined in Section 2.1.2 was followed to delimit the sample.

2.2.3. Confidence limits

Depending on the size of a given sample, the sample mean ($\overline{X}$) tended to equal the population mean (μ) as n increased. The population mean fulfills the condition shown in formula (2) with 95% probability.

$$\mu\in\left\{\overline{X}-\frac{1.96\times S}{\sqrt{n}},\overline{X}+\frac{1.96\times S}{\sqrt{n}}\right\} \tag{2}$$

Based on this property of the normal distribution, confidence limits (CL) were established using formula (3), where X is the Assigned Value, S' is the standard deviation for proficiency and n is the size of the sample. This confidence limit refers to the values that should be considered as true, since there is an acceptable error that must be assumed. Consequently, population mean (μ), the true value, lies between the upper limit (UL) and the lower limit (LL) with 95% probability (Abraira, 2002a, 2002b)

$$CL=X\pm\frac{\left(1.96\times S'\right)}{\sqrt{n}}. \tag{3}$$

2.2.4. Coefficient of variation

The variation coefficient (VC) of each sample is given by formula (4), where X is the Assigned Value and S' is the standard deviation for proficiency.

$$\mathrm{VC}=\frac{S'}{X}\times 100 \tag{4}$$

We can be sure that the Assigned Value agrees with the true value if an acceptable CV exists. The following selection criteria were used in determining the acceptable VC:

- Only pollen types whose Assigned Value (X) was over 10 were taken into account, because the VC is strongly influenced by low means.
- VC = 30 was deemed unacceptably high when referring to pollen types with an X value of between 10 and 25.
- VC = 20 was deemed unacceptably high when referring to pollen types with an X value of between 25 and 100.

- VC = 15 was deemed unacceptably high when referring to pollen types with an X value of between 100 and 500.
- VC = 10 was deemed unacceptably high when referring to pollen types with an X value above 500.

2.3. Errors

2.3.1. Absolute errors

Absolute errors (AE) were calculated as the element of the sample (χ_i) less than the "Assigned Value" (X) as shown in formula (5).

$$AE = \chi_i - X \tag{5}$$

2.3.2. Relative errors

The relative error (RE) for each pollen type provided a summary of the performance of each technician. Relative errors were obtained using formula (6), where χ_i is the value recorded by each technician, X is the Assigned Value and CL is the confidence limit value nearest to χ_i. Pollen data have a range of uncertainty below which no error is admissible; for that reason, the RE was calculated using confidence limits rather than the central reference value. RE was considered equal to zero when χ_i values lay between the two confidence limits.

$$RE = \frac{\chi_i - CL}{X} \times 100 \tag{6}$$

RE > |20%| was considered a significant error. Error was only considered significant when also: AE > |10|. The percentage of elements in which a significant error occurs is defined as percentage of error.

2.4. Analysis of sources of error

The main objective of this study is to determine the performance of staff and data quality. But the ultimate goal of assessing staff performance is to ensure that quality requirements are met, and that – should signs of poor quality be detected in the data – the problem is solved quickly and efficiently. It was essential to identify the source of error in order to address those quality issues detected. This required a number of different procedures.

All errors detected here were instrumental errors: as shown in Fig. 1, instrumental errors can be classified as mathematical errors, identification errors and counting errors. The first step was to check for mathematical errors, before going on to analyze the source of other relative errors.

2.4.1. Mathematical errors

To detect mathematical errors, relative errors were classified on the basis of total pollen–grain counts. Data were plotted as a 3D matrix plot to facilitate visual detection of the presence of mathematical error, which is detected if the same error is constantly observed for all pollen types.

The presence of mathematical error was confirmed by multivariate principal component analysis (PCA). The variables considered were the pollen concentrations determined on each sample. Each element of the sample (each technician staff) was considered as a case in PCA.

2.4.2. Identification errors and count errors

Pollen identification errors were detected as an inverse relationship between absolute errors committed in different pollen types by the same technician. That is, the presence of absolute error with magnitude = z committed by a technician staff in a pollen taxon and the presence of an absolute error of magnitude = −z made by the same technician in other pollen taxon of the same slide. Errors that are not identified as mathematical errors or as misidentifications are designated as counting errors, by discarding.

3. Results and discussion

3.1. Summary parameters

3.1.1. Assigned Value and standard deviation for proficiency

Several options were analyzed as potential summary parameters of the central reference value, including median, mean, and semi-interquartile range; the mean of the bounded sample was finally selected as the most appropriate option. This has been termed the "Assigned Value" (X). Of the range of options available as potential summary parameters for data dispersion, the standard deviation of the bounded sample was selected as the most appropriate option. This has been termed: "standard deviation for proficiency" (S').

3.1.2. Confidence limits and variation coefficients

Confidence limits were calculated in order to draw more solid conclusions. Results have been expressed in Table 1. As can be seen, the larger the standard deviation value, the greater the assumed uncertainty about the true value.

VC also is shown in Table 2. Calculation of the VC for each pollen type was essential, since a very high variation coefficient implies a high variability in counts; what we would not ensure that the Assigned Value is a true representation of the true value, therefore this taxon is not to be used to assess the individual quality of technician staff. It would therefore indicate that the overall quality of the data on this taxon is not good. High VC indicates marked uncertainty in results, which may be due to one of two factors: either staff has failed to understand the test instructions, or there is a problem with the identification of a certain pollen type. The most appropriate solution in these cases would be to take into account only the results obtained by leading experts when constructing Assigned Values to assess individual proficiency or, where there is no expert committee, not to use these cases to assess individual proficiency given the impossibility of knowing the true values of the population. The criterion for setting the acceptable VC threshold to consider the type of pollen for proficiency testing depends on the purpose of the study and on the type of data involved; the VC threshold must be set by expert consensus.

VC is greatly influenced by low sample means; the validity of the VC threshold depends on the sample mean ($\overline{X}$), a dependence which – according to Käpylä and Penttintn (1981) – can be expressed as shown in formula (7).

$$VC = \frac{100}{\sqrt{X}} \tag{7}$$

Table 1
Assigned Value (X) ± standard deviation for proficiency (S').

Sample	A	B	C
Alnus	0 ± 0	0 ± 0	0 ± 0
Amaranth.	0 ± 0	0 ± 0	0 ± 0
Betula	0 ± 0	96 ± 9	1 ± 1
Castanea	0 ± 0	0 ± 0	0 ± 0
Cupressus	156 ± 19	23 ± 4	0 ± 0
Fraxinus	1 ± 1	16 ± 8	0 ± 0
Morus	0 ± 0	2 ± 2	1 ± 1
Olea	0 ± 0	0 ± 0	3 ± 2
Pinus	1 ± 1	167 ± 20	7 ± 2
Plantago	0 ± 0	1 ± 1	18 ± 4
Platanus	0 ± 0	142 ± 18	114 ± 12
Poaceae	1 ± 1	7 ± 3	11 ± 1
Populus	86 ± 14	43 ± 8	0 ± 0
Quercus	0 ± 0	96 ± 16	91 ± 3
Rumex	0 ± 0	0 ± 0	24 ± 3
Urtica	18 ± 9	7 ± 3	4 ± 2
Total	273 ± 32	618 ± 54	277 ± 28

Table 2
Confidence limits and variation coefficients (VC). Upper limit (UP), lower limit (LL), slide A (A), slide B (B) and slide C (C). Variation coefficients = * when $X<10$.

Sample	A			B			C		
	UL	LL	VC	UL	LL	VC	UL	LL	VC
Alnus	0	0	*	1	0	*	0	0	*
Amaranth.	0	0	*	0	0	*	0	0	*
Betula	0	0	*	0	0	*	1	1	*
Castanea	0	0	*	100	93	10	0	0	*
Cupressus	163	148	12	25	21	19	0	0	*
Fraxinus	2	1	*	20	13	50	0	0	*
Morus	0	0	*	3	2	*	1	1	*
Olea	0	0	*	0	0	*	3	2	*
Pinus	1	1	*	175	159	12	8	6	*
Plantago	0	0	*	1	0	*	19	16	21
Platanus	0	0	*	149	135	13	119	109	11
Poaceae	1	0	*	8	6	*	11	10	13
Populus	91	80	17	47	40	19	0	0	*
Quercus	0	0	*	102	90	16	95	86	12
Rumex	0	0	*	0	0	*	25	23	12
Urtica	21	14	49	8	5	*	5	4	*
Total	285	260	12	639	597	9	288	266	10

The relationship between the VC and the sample mean ($\bar{X}$) for the results obtained here is shown in formula (8) and plotted in Fig. 2.

$$\mathrm{VC} = 102.7 * \overline{X}^{-0.45} \tag{8}$$

As Table 2 shows, the VCs were generally acceptable, the only exceptions being those obtained for *Urtica* pollen in slide A and for *Fraxinus* pollen in slide B. Although one can hazard a guess at the cause of these high VCs, these pollen types should clearly not be taken into account for the proficiency test: external factors influenced *Fraxinus* pollen counts in sample B, while the high VC for the *Urtica* pollen count may be due to a misunderstanding of the technical instructions; in some cases all *Urtica* pollen types were taken into account, while in other cases only *Urtica membranacea* pollen or *Urtica*/*Parietaria* pollen was used.

The VC of samples with an AV greater than 20 was 13%, while the VC of data with an AV greater than 150 was 10%. These data confirm generally good performance by technicians.

3.2. Errors

Like the CV, error is strongly influenced by the sample mean, as noted by a number of authors (Comtois et al., 1999; Sikoparija et al., 2011). Errors were considered significant when RE > |20%| and AE > |10|. However, the acceptable threshold for RE should be taken by consensus among experts. Comtois et al. (1999), in a survey of the performance of aerobiology experts, highlighted the uncertainty regarding the error to be considered acceptable.

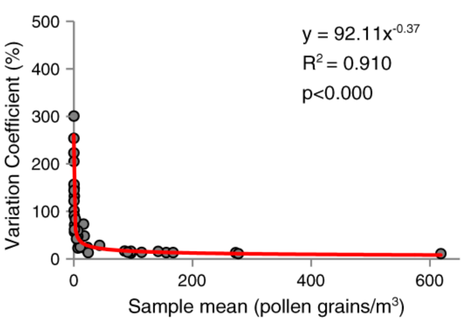

Fig. 2. Variation coefficients vs. sample mean.

Here, technicians made significant relative errors in only 3.5% of elements, thus confirming good overall performance.

3.3. Detection of error sources

As indicated earlier, this type of testing detects instrumental errors (Fig. 1); these may be technician errors (in pollen recognition or counting) or mathematical errors. Statistical testing can forcefully assert the presence of an error, and can even quantify its magnitude, as shown here. However, statistical tests cannot be used to detect the source of error. Below have been identified the source of error. This lay the basis for future specific remedies in order to enhance data quality. In case of finding any general problem in the quality of data, all members of the study must be informed so they can remedy it. Also, a report must be sent individually to each participant indicating the cause of their mistakes. Appropriate corrective measures must be implemented to solve the problem.

3.3.1. Mathematical errors

In some cases, a mathematical error was apparent in the results: technicians recorded very low counts for all pollen types, suggesting an error in applying microscope correction factors. In such cases, results contained an internal bias. Mathematical error in the results obtained by four technicians (9, 12, 15 and 17), all of whom recorded very low mean concentrations for all taxa, is apparent in the diagram in Fig. 3.

The internal bias shown in Fig. 3 was confirmed by multivariate principal component analysis (PCA), Fig. 4, on the assumption that any technician displaying a repetitive pattern on all counts should be clearly reflected.

3.3.2. Identification errors and counting errors

Only one significant identification error was found between *Populus* and *Platanus* taxa, and there were no counting errors. Certain statistical parameters for sample A as analyzed by several technicians (only *Populus* and *Platanus* pollen types) are shown in Table 3. Technician 22 displayed high relative errors in both pollen types: an absolute overcount error of 47 for *Platanus* pollen and an absolute undercount error of 40 for *Populus* pollen. This fact suggests an identification error between *Platanus* and *Populus* pollen grains.

4. Conclusions

In general, data quality is good. The average VC was 13% in samples where $X>20$; however, the VC is strongly dependent on the sample mean, and using only samples with $X>150$, the VC was 10%. Two pollen types showed unacceptable VC. Significant relative errors were found

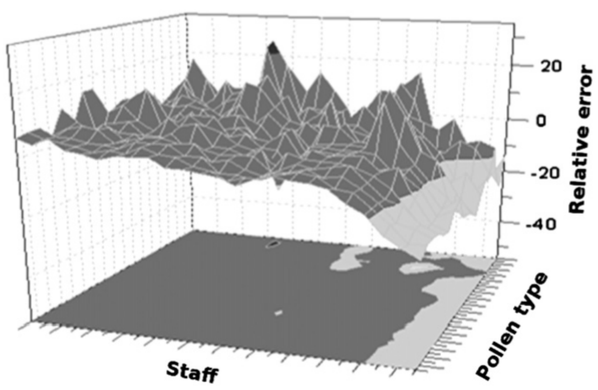

Fig. 3. Absolute errors matrix 3D plot.

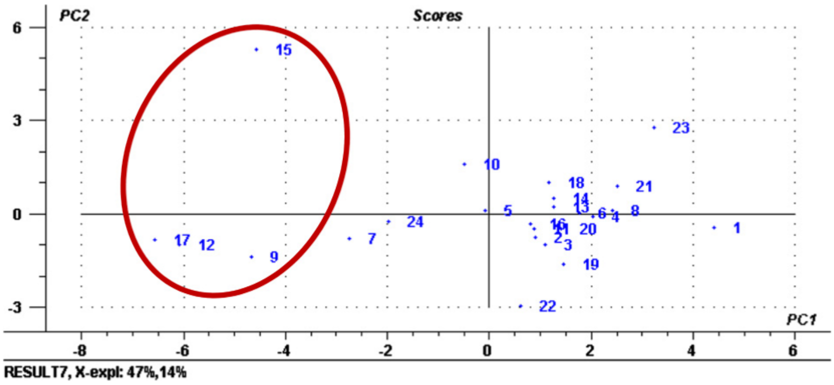

Fig. 4. PCA of all pollen concentrations.

Table 3
Some summary parameters of several staffs. RE (relative error), AE (absolute error).

Staff	RE	RE	AE	AE	Count	Count
	Platanus	*Populus*	*Platanus*	*Populus*	*Platanus*	*Populus*
23	0	5	0	2	149	49
5	0	9	0	4	137	51
3	0	3	0	1	146	48
25	−2	0	−3	0	132	41
22	33	−100	47	−40	196	0
7	−1	−9	−2	−4	133	36
1	−4	0	−5	0	130	44
16	−1	1	−1	0	134	47
2	0	−2	0	−1	136	39

only for 3.5% of observations. Errors have been detected in some counts of 5 staffs. The procedures tested here for identifying error sources have enabled the appropriate corrective measures to be implemented, and the problem has been solved.

Our results marked a new step forward in quality control programs, thanks to the development and implementation of a new, effective error-correction method as part of a QC program to be used in proficiency testing in aerobiology. This exercise has also served to confirm the good overall performance of REA technician staff.

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